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NORMAL AND ABNORMAL BLOOD COAGULATION: A REVIEW

BY

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(RECEIVED FOR PUBLICATION, MARCH 16, 1948)

Introduction

As one of the lesser problems of physiology, the nature of blood coagulation has received more than its share of investigation. There is now an insurmountable mass of literature dealing with its various aspects, contributed over the greater part of a century by some of the foremost experimentalists of their time, and embodying theories, even whole schools of thought, that have produced little but acrimony and confusion. The reasons for this activity are not at once apparent, though no doubt there is something provocative to the curious mind in the spontaneous conversion of fluid blood to solid clot. More attractive, perhaps, is the ease with which experiments in blood coagulation can be devised and carried out. The requirements for such work in technique, apparatus, and even in time are seemingly not exacting. The real or imaginary components of a theoretical mechanism can be separated to the investigator's satisfaction with little difficulty and allowed to interact in endless permutations and combinations. Each experiment suggests another; always the intangible solution seems just within reach and the experimenter is led deeper and deeper into his own, often unjustified, interpretations of his findings. Thus it is that elaborate theories have grown up which, aided by the confusion of rival terminologies, have so obscured and entangled basic facts that these are now scarcely recognizable.

Within the last decade this hitherto rather academic problem has taken on a more practical aspect; the discovery of vitamin K and the use of anticoagulants to reduce the danger of post-operative thrombosis, both advances of fundamental importance, have demanded the reliable quantitative assay of coagulation factors. This development has necessarily clarified some of the obscurities, but it has thrown a strain on accepted theory, which is now showing signs of undergoing considerable modification. It seems, therefore, that some review or rather "interim report" of the problem might be desirable. Though the present communication is an attempt to provide this, it is no more than an outline and in no way claims to be comprehensive.

*Aided by a grant from the Medical Research Council.

The Function of Blood Coagulation

Haemostasis.—Before discussing the mechanism of blood coagulation, it would be well to consider briefly its functional significance. Its most obvious use is concerned with haemostasis. The fact that wounds which have ceased to bleed are filled with blood clot naturally suggests that the clotting of the blood has arrested the haemorrhage. This is supported by the fact that in conditions with defective blood clotting there is a tendency for patients to bleed for long periods from even slight injuries. Coagulation is not, however, the only factor concerned in this tendency, since severe haemorrhagic states may exist despite a normal clotting mechanism. The part played by coagulation has been discussed in detail by the author (Macfarlane, 1941), who concluded that after injury there is a period of bleeding from cut vessels which is succeeded by a period of haemostasis lasting for about an hour and which is due primarily to the contraction of the damaged vessels. These vessels then dilate, but recurrence of the haemorrhage is prevented by the firm coagulation of the blood that had filled the wound during the initial haemorrhage. Normal coagulation, therefore, is the essential complement to the vascular contraction (and perhaps normal platelet function) which initiates haemostasis. Either factor without the other is useless. This "two stage" hypothesis of haemostasis and the two main abnormalities which, in the author's view,

cause the haemorrhagic states is illustrated diagrammatically in Fig. 1 (Macfarlane, 1945). It will be seen that this hypothesis in no way lessens the supposed importance of coagulation in the mechanism of haemostasis, but regards it as an interlocking part of a more complex system.

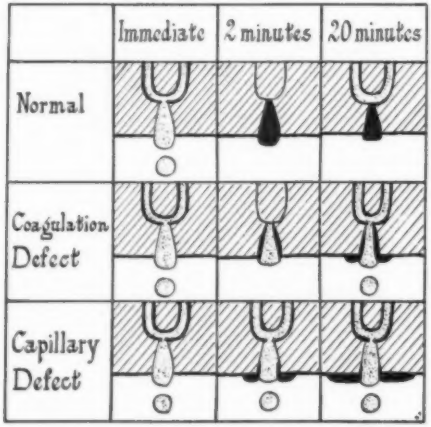


FIG. 1.—Diagram illustrating the time relationship of normal capillary contraction, dilatation, and blood coagulation, and the two main defects that may occur. A wound of the skin surface is shown in section, injuring a capillary loop. Fluid blood is represented by the dotted areas, blood clot by solid black, and the detached "drops" indicate active haemorrhage. (Macfarlane, 1945. Reproduced by permission of the publishers of the *Proc. roy. Soc. Med.*)

Resistance to bacterial invasion.—The inflammation accompanying local bacterial infection is usually associated with plasma transudation into the tissue spaces. Coagulation of this oedema fluid takes place and the fibrin so formed serves as a barrier more or less effective in preventing the spread of infection through the adjacent tissues. It has been found experimentally (Thuerer and Angevine, 1947) that impairment of coagulation by administration of dicoumarin greatly reduces the minimal lethal dose of pathogenic organisms injected subcutaneously into rabbits. It may be that the characteristic invasiveness of the haemolytic streptococci as compared with the localization of staphylococcal infection is due to the fibrinolytic action of the former as against the coagulase produced by the latter. It has been pointed out by Robb-Smith (1945) that a feature of gas-gangrene due to *Cl. welchii* is the almost complete absence of local fibrin formation, and in no other infection is tissue invasion so rapid. The fact that in most infections there is a marked rise in the fibrinogen content of the blood may be related to a defence reaction of this type.

Healing.—The importance of a fibrin network in the process of repair of wounds has often been suggested, but actual proof is not available. It is a fact, however, that in haemophilia the healing of even minute wounds is extremely slow if haemorrhage is taking place. It is no unusual experience for a patient to ooze blood for weeks from a small abrasion that normally would be healed completely in a few days. The continued bleeding itself may prevent the reparative processes from making headway, but it is also possible that these require the presence of preformed fibrin as a scaffold for the new network of collagen and capillaries.

Metabolism.—The mechanism of blood coagulation is extremely elaborate, requiring the interaction of many factors. Some of its protein constituents, such as fibrinogen and prothrombin, are normally present in considerable excess of what is required for haemostatic efficiency. This prodigality has seemed to a number of authorities to suggest that coagulation is a result of a constantly operating metabolic process that has been adapted to fulfil the secondary purpose of haemostasis. In support of this, it appears that both fibrinogen and prothrombin are consumed with considerable rapidity in the absence of any obvious clotting activity, though some of the evidence from which this is inferred is questionable, as will be seen later. The fact that proteolysis appears to be in some way connected with the coagulation mechanism also suggests that the latter may be concerned in normal protein metabolism.

Finally, despite these speculations it can be said that blood coagulation is not essential to life. Several patients have been observed to lead a relatively normal existence though they have no fibrinogen in their blood.

Normal Blood Coagulation

Historical.—While it is outside the scope of this article to give more than an outline of the historical background to the present views on blood coagulation, such a background assists the visualization of the problem in its proper perspective.

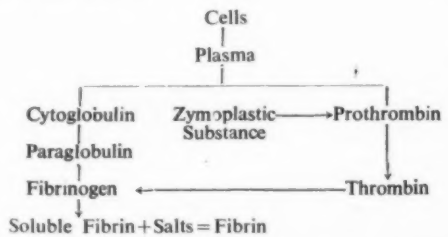
Malpighi (1666) was one of the first to consider the nature of the solidity of clotted blood. He found that after a clot was washed in water a mass of fibres remained. In 1735 the French surgeon Petit observed that coagulation was an attribute of the clear, fluid part of the blood, not of the cells, an observation that was extended by Hewson (1770), who called this fluid "coagulable lymph" and showed that clotting could be prevented by cold, or the presence of salts.

Chaptal (1797) applied the appropriate name, "fibrin," to the fibres constituting the clot. The nature of the clotting process then began to evoke considerable discussion. Hunter (1794) maintained that it was a "vital" action of the blood analogous to the contraction of a living muscle. Others considered that it was chemical in nature, and depended on such changes as contact of the blood with the air, cooling, or stasis.

In 1835 Buchanan made a series of observations that anticipated the discovery of thrombin and thromboplastin. He showed that the clotting of "coagulable lymph," in this case hydrocele fluid, was accelerated by the addition of the washings of a blood clot or of leucocytes. He compared the action of these substances to that of rennin on milk, thus founding the concept of a reaction between a precursor of fibrin and a ferment-like agent generated in the blood during clotting; but his work received little recognition until it was resuscitated by Gamgee (1879). The development of a clearer understanding of the coagulation mechanism was hampered for a while by the popular theory, propounded by Richardson (1858), that blood clotted when it left the vessels because the ammonia it contained during life was then able to escape. This tenacious fallacy was finally exploded by Lister (1863), who performed a series of experiments that are a model of ingenuity and precision. A few years before this Denis (1859) had been able to separate the fluid precursor of fibrin, calling it "plasmine," a name changed to the more descriptive "fibrinogen" by Hammarsten (1877).

The scene was now set for Schmidt (1892) to present his conception of a chain of reacting factors culminating in the conversion of fibrinogen into fibrin by a ferment called thrombin, a theory that has held the stage ever since. Schmidt recognized that thrombin was not present in the circulating blood, and he postulated an inactive precursor, prothrombin, that was activated by zymoplastic substance, considered, even at that early stage, to be lipoidal in nature.

The hypothesis of coagulation, as it stood at this time, is illustrated by the following diagram quoted by Quick (1942):

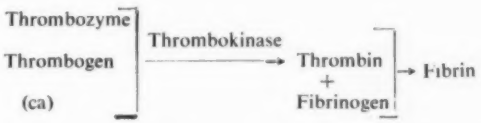


It remained for Arthus and Pagès' (1890) discovery of the essential part played by ionized calcium in the activation of prothrombin to complete the basis for the modern conception of the coagulation mechanism. This was restated in a more simple form by Morawitz (1905), who considered that clotting involved two stages and four essential components, thus:

1. Prothrombin + Ca + Thromboplastin = Thrombin
2. Fibrinogen + Thrombin = Fibrin

This, the so-called classical theory, has been pre-eminent up to recent times. This is not to say that other theories have not been put forward; many indeed are the spirited, if sometimes bizarre, heresies that have arisen, so that at times the number of theories has exceeded the number of propounders (Wöhlisch, 1929), but, particularly in the United States and in this country, no attack on this "4-factor" theory has been taken seriously.

Two unorthodox views formulated at the beginning of this century must, however, be considered. The first is that produced by Nolf (1908) and still vigorously defended by him (1945). In Nolf's view, coagulation requires, in addition to calcium, the interaction of *four* protein-like factors, all of which are present in the plasma. These factors are: thrombokinase (thromboplastin); thrombozyme; thrombogen; and fibrinogen. The thrombokinase of the plasma is a lipoprotein, having the same properties as tissue thrombokinase, and accelerating, in the presence of calcium, the reaction between thrombozyme and thrombogen. These two factors together constitute what was usually called "prothrombin" and which is therefore not a simple substance but a mixture. Neither thrombogen or thrombozyme alone is capable of generating thrombin without the presence of the other, and thrombin is regarded as a product of their union, since its formation coincides with their disappearance. The theory can be illustrated diagrammatically thus:



According to Nolf, the thrombozyme-thrombogen complex is responsible not only for the coagulation of fibrinogen but also for fibrinolysis, particularly if the thrombozyme is in excess of its normal ratio to thrombogen. The lifelong work on blood coagulation of this investigator has received far less attention than it deserves. This is due in part to the rather obscure presentation of his admittedly complex conception and the involved experimental evidence on which it is based. It is largely due, however, to the fact that many modern workers have not troubled to read his published material. As will be seen later, some aspects at least of his theory are receiving unsuspected confirmation.

Another supporter of a "5-factor" theory was Bordet (Bordet and Delange, 1912; Bordet, 1920). He postulated an inert precursor (proserozyme) of what is usually termed prothrombin, which was activated to serozyme by contact with a foreign surface

in the presence of calcium. Serozyme then combined with cytozyme (thromboplastin) to form thrombin, thus :

1. Proserozyme —(contact+Ca)→ Serozyme
2. Serozyme+Cytozyme → Thrombin
3. Thrombin+Fibrinogen → Fibrin

Bordet's theory is based on a number of interesting experiments which may have a significant part in any modern re-orientation of the classical theory, though Bordet's own interpretations may not always be acceptable.

These earlier theories in their preoccupation with clotting did not explain adequately the fluid state of the normal circulating blood. Lister (1863) had emphasized the importance of contact action in the inception of blood coagulation, and it has already been stated that other workers recognized the importance of admixture with tissue fluid containing thromboplastin, or its release following the disruption of cells or platelets when blood is shed. The absence of contact with a foreign surface, however, is not a sufficient explanation of the inherent stability of blood in the circulation, since in a variety of pathological and traumatic conditions both vessels and tissues may be so injured as to permit contact action and the passage of thromboplastin into the blood stream.

It was Howell (1910, 1911, 1935) who first emphasized the possible importance of anticoagulant substances, and who laid the foundations for the idea of an equilibrium between such anticoagulants and the coagulant factors that come most strongly into play when blood is actually shed. He at first postulated an antiprothrombin that was neutralized by thromboplastin. Later he assumed that the newly discovered anticoagulant substance heparin (Howell and Holt, 1918) was identical with this factor. His theory at that time could be illustrated as follows:

1. (Prothrombin − Heparin)+Thromboplastin→ Prothrombin
2. Prothrombin+Ca → Thrombin
3. Thrombin+Fibrinogen → Fibrin

Later work suggests that heparin is mainly an antithrombin, but the concept of anticoagulant factors essential for the preservation of normal blood fluidity still maintains its place in present-day explanations of the coagulation mechanism.

Among workers in this country whose investigations advanced the international knowledge of this subject during the early part of the century, Mellanby (1909) and Pickering (1928) are foremost. The former perfected the technique of separation and study of active fractions of the coagulation mechanism, and by his study of their properties found a solid basis for much modern work (Mellanby, 1930, 1933). Pickering contributed his exhaustive monograph on the subject, together with his synthesis of the divergent and discordant views of his predecessors.

The Factors of the Classical Theory

Before discussing the recent events that have resulted in a modification of the earlier acceptance of the classical theory, some of the well-established facts relating to the familiar components of this system should be reviewed.

Fibrin.—The end-result of normal mammalian blood coagulation is the formation of the fine three-dimensional network of fibres that entangles the formed elements of the blood. Under certain circumstances, principally those involving changes of *p*H, application of heat, or long storage, clotting produces a structureless gel rather than a reticulum, but this, though of theoretical interest, might be regarded as an artifact.

Examination of the minute structure of the fibrin threads by polariscopic (von Dungern, 1937), radiological (Herman and Worschitz, 1935), or electron-microscopical means (Wolpers and Ruska, 1939; Hawn and Porter, 1947) has suggested that they are composed of needle-like micelles packed lengthwise into bundles. When freshly formed the threads are extremely adhesive, sticking to each other, to the platelets and cells of the blood, to the tissues, and to certain foreign surfaces—a property to which the blood clot owes its haemostatic efficiency.

A curious reaction of freshly coagulated fibrin is that of retraction or, more properly, contraction. The normal blood clot will contract to about 40 per cent of its original volume, squeezing out serum mixed with a small proportion of red cells. Plasma clots will contract to 10 per cent or less of their original volume (Macfarlane, 1939). This activity is reduced naturally in those conditions in which there is thrombocytopenia and can be inhibited artificially by heating to 47° C. or by removing the platelets (Macfarlane, 1938a). The significance of retraction from the point of view of haemostasis is not clear, but there is no doubt that the contracted clot is more solid and elastic than when it is newly formed.

Fibrin is soluble in acids and alkalis, and is digested by proteolytic enzymes. After such solution it cannot be made to re-form into its original reticulum. If it is kept sterile, the fibrin of whole clotted blood will remain intact for days or weeks under normal conditions, but in certain circumstances it undergoes rapid dissolution or fibrinolysis, a process now known to be due to the activation of a proteolytic enzyme of the plasma, and discussed by Macfarlane and Pilling (1946a).

It has been suggested that fibrin may exist temporarily in a soluble form in the immediate pre-clotting stage. This hypothetical precursor of fibrin has been called profibrin by Apitz (1937), and its

existence is accepted by Astrup (1944) and Owren (1947a). Apitz (1939) believes that a sticky film of profibrin absorbed on to the platelets during coagulation causes their agglutination, an explanation recalling the earlier suggestion of Maltaner and Johnston (1921) that fibrinogen was necessary for the "conglutination" of erythrocytes and bacteria.

Fibrinogen.—Fibrinogen may be described as the fraction of the plasma protein which is coagulable by thrombin. It has the greatest molecular weight of the plasma proteins, a recent estimate being 441,000 (Nanninga, 1946). It is also the most labile, being precipitated by heating to 47° C. or by half saturation with sodium chloride or quarter saturation with ammonium sulphate. Its iso-electric point is *p*H 5.5 (Nordbö, 1927), and it is precipitated by bringing the *p*H to this point after reduction of electrolyte by preliminary dialysis or dilution with distilled water.

Wöhlisch and Clamann (1932) and Wöhlisch and Kiesgen (1936) have presented evidence that the molecule of fibrinogen is long and thread-like, this conclusion being in accord with the evidence of the miscellar structure of fibrin already mentioned.

It is generally assumed that fibrinogen is formed by the liver. The evidence for this is as follows: Nolf in 1905 showed that exclusion of the liver from the circulation of dogs resulted in a rapid and profound fall in the blood fibrinogen. This observation was supported by other workers, including Jones and Smith (1930), who observed a similar fall after hepatectomy. Destruction of liver function by the administration of chloroform or phosphorus will also produce the same result (Foster and Whipple, 1921) accompanied, in the case of the former agent, by a decrease in prothrombin as well (Smith, Warner, and Brinkhous, 1937).

In such experiments it is assumed that the fall in fibrinogen is due to the cessation of production by the damaged or excluded liver, the normal rate of fibrinogen utilization being presumably very high. Against this, however, must be set certain objections. The most serious is that substances such as chloroform induce intense fibrinolytic activity, a fact well recognized by workers on this subject. Fibrinolysis was also observed by Macfarlane and Biggs (1946) to occur after any operative procedure, and it has also been shown to occur in severe liver damage. It might be argued, therefore, that the disappearance of fibrinogen in these cases was due to an increased rate of destruction rather than to deficient production (Macfarlane and Biggs, 1948). The second objection involves the normal rate of utilization of fibrinogen. Admittedly in the experimental animals described the rate of utilization may have been higher than normal as a result of haemorrhage. In human beings there is little direct evidence of the normal rate of usage apart from the observation of Pinniger and Prunty (1946) that fibrinogen transfused into a patient with congenital absence of fibrinogen was still present in a detectable amount after ninety-six hours. If this reflects the rate of utilization in normal persons, removal of the source of supply would not have resulted in a detectable drop in the course of the few hours' duration of an acute experiment. The whole question thus requires re-examination.

Most investigators consider that fibrinogen is a homogeneous protein, and that they are able to obtain it in a state of considerable purity by fractionation methods. Some, however, have postulated the existence of different forms of fibrinogen, a view which is now receiving increasing experimental support. As long ago as 1889 Wooldridge regarded fibrin as being derived from the combination of two components, fibrinogen A and fibrinogen B, neither being identical with the fibrinogen separated in the laboratory from plasma, which he regarded as an artifact. Recent work by Nolf (1947) suggests, however, that Wooldridge's fibrinogen A is in some respects similar to the classical thromboplastin, but there are interesting points of difference. Wertheimer and others (1944) have shown that on storage the fibrinogen of plasma separates into a more labile fraction that is precipitated by cooling and a less labile fraction remaining in solution. The proportion of less labile fibrinogen is reduced in liver disease and increased in pregnancy or after haemorrhage.

A very interesting contribution is that of Lyons (1945a, b), who suggests, on the basis of coagulability by naphthoquinone, that there are two forms of fibrinogen—fibrinogen A, which exists in fresh plasma and normal blood, and fibrinogen B (these names are not to be identified with those of Wooldridge), which occurs in certain pathological states and develops on storage of blood and during the clotting of fibrinogen with thrombin. The significance of this work will be discussed later.

Thrombin.—Thrombin is the factor which, though absent from the normal circulating blood, develops during the process of coagulation and on separation can be shown to be capable of clotting fibrinogen. Though it is doubtful if pure thrombin has been obtained or is even obtainable, preparations having very great activity relative to dry weight can be produced with little difficulty, and such preparations can clot several hundred times their own weight of fibrinogen (Eagle, 1935a;

Seegers, 1940 ; Astrup, 1944). Astrup (1944) and his colleagues have shown that thrombin is an albumin-like protein, with a molecular weight of about 75,000. The phosphorus content of Astrup's preparations was too low to be detected by chemical means, a finding that agrees with that of Chargaff and others (1940), who followed the generation of thrombin using a radioactive phosphorus isotope. The calcium content is also negligible (Ferguson, 1937). Thrombin is heat-labile, being destroyed by heating to 60° C., and beginning to lose activity at 40° C.

Lyons (1945b), having found that thrombin digested with trypsin gives a colour reaction characteristic for this substance, maintains that thrombin contains a naphthoquinone group.

Reaction of thrombin with fibrinogen.—The earlier authorities regarded thrombin as an enzyme acting specifically on fibrinogen. Mellanby (1933) called thrombin "thrombase" and thought that it split the fibrinogen molecule into fibrin and a soluble globulin. There was a temporary setback to the enzymatic view, largely due to the work of Howell (1910), from which it appeared that thrombin was consumed quantitatively during the conversion of fibrinogen to fibrin, a fact possibly explained by absorption on to the newly formed fibrin and the presence of antithrombin. Howell's contention was to some extent supported by Hekma (quoted by Pickering, 1928), who thought that thrombin acted as an agglutinin of fibrinogen in the ordinary immunological sense. Pickering (1928) argued against this, pointing out that thrombin is almost inactive at low temperatures, is thermolabile, and soon loses its activity on storage, while agglutinins are more active at low temperatures, are heat stable, and retain their activity for years.

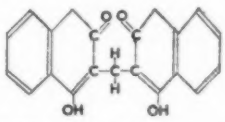
Since more recent work, already quoted, has shown that thrombin can convert at least 600 times its own weight of fibrinogen into fibrin (Astrup, 1944) and that the molecular weight of thrombin is probably not less than one-tenth that of fibrinogen, it seems unlikely that any stochiometric combination of thrombin and fibrinogen can occur, and it seems as if the enzymatic action of thrombin is more probable.

The nature of this enzymatic action is not known. Mellanby's (1933) view that the fibrinogen molecule was split into a soluble and an insoluble fraction is unlikely to be correct, since Jaques (1938) found that the fibrinogen-nitrogen lost during clotting reappeared quantitatively as fibrin-nitrogen. However, the supposition that the process was, in some way, a proteolytic one had some circumstantial support in that certain proteolytic agents such as papain (Eagle and Harris, 1937) and the proteolytic venoms of *Echis carinata* (Barratt, 1920) and other snakes (Eagle, 1937) can coagulate fibrinogen. Moreover, it was argued that thrombin preparations were often fibrinolytic as well as fibrin-forming, this suggesting that coagulation was merely the prelude to lysis (Nolf, 1938). More recently, however, there is strong evidence to show that the clotting action of thrombin is distinct from any fibrinolytic action which certain preparations may have and which is almost certainly due to contamination with plasmin, the proteolytic enzyme of the plasma (Seegers, 1940 ; Ferguson, 1943 ; Macfarlane and Pilling, 1946a).

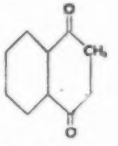
In 1941 Baumberger made the fruitful suggestion that thrombin contributed to the formation of fibrin by potentiating a chemical linkage between fibrinogen molecules. He considered that the SH groups known to be present in the fibrinogen molecule might be oxidized by thrombin, so that an S-S linkage between fibrinogen molecules could occur, with consequent construction of the fibrin lattice, thus :

$$2\,(\text{Fibrinogen—SH}) \xrightarrow{\text{Thrombin}} \text{Fibrinogen—S-S—Fibrinogen}$$

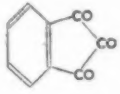
This conception has been extended by Lyons (1945a and b). It has already been stated that he believes that the thrombin molecule contains a naphthoquinone group, and that this substance is able, by itself, to clot a certain form of fibrinogen known as fibrinogen B. He explains the previous observations that ninhydrin will clot fibrinogen (Chargaff and Ziff, 1941) on the basis of the similarity of the structure of this substance to the naphthoquinones (Fig. 2). The failure of others to



3, 3′-Methylene-bis-(4-hydroxycoumarin)



2-Methyl-1,4-naphthoquinone



Ninhydrin

FIG. 2.—Structural formulae of certain substances active in the coagulation mechanism.

obtain coagulation of fibrinogen by naphthoquinone is due to the use of fibrinogen A in their experiments. Lyons suggests that in its native form fibrinogen has its thiol groups blocked, this form constituting fibrinogen A. On storage, or in certain pathological states, fibrinogen A may become partly converted to fibrinogen B, in which the SH groups are free. Such fibrinogen is coagulable by ninhydrin and the naphthoquinones. During natural coagulation fibrinogen A is acted upon by a component of thrombin (thrombin A), the thiol groups being freed. The fibrinogen B thus formed is then clotted by the naphthoquinone component of thrombin (thrombin B) thus:

1. Fibrinogen–SH (blocked)+Thrombin A
(Fibrinogen A) ↓
2. Fibrinogen–SH+Thrombin B (or naphthoquinone)
(Fibrinogen B) ↓
3. Fibrinogen–S-S–Fibrinogen (Fibrin gel)

This work, which is supported by a number of points of evidence, is of considerable interest and, if confirmed, of obvious practical and academic importance.

Laki and Mommaerts (1945) have also postulated a two-stage reaction in the conversion of fibrinogen to fibrin, but have been less precise as to the mechanism.

Prothrombin.—Prothrombin is the inactive precursor of thrombin, and, as such, is present in the circulating blood. It has the physical and chemical attributes of a globulin (Astrup, 1944), being precipitated at *p*H of 5.6 and by half saturation with ammonium sulphate. Like thrombin its activity is destroyed by heating to 60° C. It is strongly adsorbed by such substances as $BaSO_4$ (Dale and Walpole, 1916), $Ca_3(PO_4)_2$ (Bordet and Delange, 1914), $Mg(OH)_2$ (Fuchs, 1929), and $Al(OH)_3$ (Quick, 1935a). It is also adsorbed by Seitz or Berkfeld filters. It is destroyed by proteolytic enzymes (Eagle and Harris, 1937).

The evidence that prothrombin is produced by the liver is similar to that purporting to show that fibrinogen has the same origin, that is, upon the observation of prothrombin deficiency arising after chloroform poisoning (Smith and others, 1937) or partial hepatectomy (Warner, 1938). Since prothrombin is destroyed by proteolytic enzymes and the experimental procedures described are potent activators of the proteolytic system of the blood, the same objections to these conclusions can be made as were discussed in relation to the site of origin of fibrinogen.

Vitamin K.—The story of the discovery of vitamin K and of its essential part in prothrombin production by the body now forms a familiar page in the history of fundamental medical advancement. It is instructive and stimulating to study the rapid unravelling of threads leading to an understanding of a whole series of haemorrhagic disorders that resulted from the brilliant and often independent contributions produced in quick succession. In 1934 Dam and Schönheyder reinvestigated the haemorrhagic disease that develops in chicks fed on a synthetic diet, and Dam (1935) decided that it was due to the absence of a factor (coagulation-vitamin or vitamin "K") required for the proper clotting of the blood. In the same year Halbrook (quoted by Quick, 1942) showed that the condition was cured by the administration of alfalfa (lucerne) or its ether extract, a finding confirmed and expanded by Almquist and Stokstad (1935). Further investigations by Dam and others (1936) showed that the haemorrhagic disease was due to a prothrombin deficiency, a conclusion reached independently by Quick (1937), who also showed that the prothrombin level could be restored to normal by feeding alfalfa. Quick (1935b) had already discovered the prothrombin deficiency in obstructive jaundice and he (1937) was not slow to predict that this might be due to a deficiency of the new (fat-soluble) vitamin because of deficient adsorption in the absence of bile salts from the intestine. He was able to substantiate this prediction (1938a) by actual clinical observations, which were confirmed by Warner and others (1939a) and by Butt and others (1938).

Efforts were now made to isolate the new substance. It was found that it is widely distributed in plants, particularly in green leaves (Dam and Glavind, 1938), and that bacteria can synthetize the vitamin, so that its production in the animal intestine may be an important factor in its natural assimilation (Almquist and others, 1938). Scientific methods of assay (Almquist and Klose, 1939a) based on Quick's (1935b) prothrombin-time method greatly assisted this progress in the study of the distribution and synthesis of vitamin K. As regards the chemistry of vitamin K, the principal work was carried out by Dam and his co-workers on the one hand and Almquist's group on the other (see review by Almquist, 1941). The structure of two naturally occurring substances with vitamin-K activity were quickly determined, both being derivatives of 1,4-naphthoquinone. Almquist and Klose (1939b) found that phthiocol, a compound isolated from tubercle bacilli, had vitamin-K activity and was also a 1,4-naphthoquinone derivative. It was then almost simultaneously discovered by four separate groups of workers (Almquist and

Klose, 1939b; Ansbacher and Fernholz, 1939; Fieser and others, 1939; Thayer and others, 1939) that the comparatively simple synthetic substance 2-methyl-1,4-naphthoquinone had even greater vitamin-K activity than the naturally occurring compounds. Since that time a number of derivatives of this substance with the added advantage of solubility in water have been produced commercially under different trade names, and vitamin K has taken its place among the factors essential for life.

Its mode of action in the production of prothrombin is at present unknown, though the observations of Lyons (1945b) suggest that it is an integral part of the thrombin molecule. It has no effect on prothrombin activity *in vitro* and its administration in cases of hypoprothrombinaemia is effective only if the function of the liver is relatively normal. In the latter case the rise in prothrombin following vitamin-K administration is very rapid, a tenfold increase occurring within twelve hours in some patients (Quick, 1942).

Calcium.—It is now undisputed that ionized calcium is required for the conversion of prothrombin to thrombin, but that the latter can act on fibrinogen with unimpaired effect in the absence of calcium or in the presence of decalcifying agents such as oxalate or citrate.

The calcium of the blood-plasma is present in two forms; the diffusible ionized calcium and the non-diffusible protein-bound fraction, which is usually thought not to take part in the coagulation process. Removal of free calcium by precipitating it as the oxalate, or suppression of ionization by the addition of citrate, will inhibit the normal clotting process indefinitely. It is significant, however, that if clotting is to be prevented at least three times as much oxalate must be added to plasma as the calculated amount necessary to precipitate all the available calcium (Vines, 1921; Scott and Chamberlain, 1934; Quick 1940). This finding is difficult to understand unless it is supposed that there is a competition for available calcium by the coagulation factors on the one hand and the decalcifying agent on the other, it being necessary to have an excess of the latter to deprive the former of calcium by mass action.

Ferguson (1937) supposed that an intermediate prothrombin-calcium-thromboplastin complex was formed during clotting and that this complex could be inactivated by decalcification. Quick (1940) also postulates a prothrombin-calcium complex, from which calcium has to be removed before the decalcifying agent can prevent coagulation. He has also provided evidence (1947b) that there is a calcium co-factor associated with, but distinct from, prothrombin. Greville and Lehman (1944) have studied the interesting cation antagonism affecting this part of the clotting mechanism, in which certain ions, particularly those of magnesium, antagonize the effect of calcium.

The amount of calcium required for effective coagulation has a wide optimum value, ranging from 0.025 to 0.0025 molar concentration. Above and below this level coagulation is depressed and finally inhibited (Quick, 1942).

Thromboplastin.—Although blood which is collected without contamination with tissue fluid and which is then kept in a container with a "neutral" (non-water-wettable) surface will not clot, it can be made to do so with great rapidity by the addition of a small quantity of the watery extract of almost any tissue. It can therefore be assumed that prothrombin, even in the presence of calcium, is inactive, but that it can be activated by a factor or complex of factors present in the tissues. This agent has received various names, such as cytozyme, thrombokinase, thromboplastin, zymoplastic substance, or simply kinase. It occurs in most tissues, but brain, lung, thymus, testes, and platelets yield the most active preparations, and it can be demonstrated in human saliva (Bellis and Scott, 1932).

In discussing the nature, properties, and modes of action of thromboplastin a major difficulty arises which is by no means limited to this particular aspect of the coagulation problem. This is the probability that "thromboplastin" has more than one component, and that different substances, claimed by various workers to be thromboplastin in a state of relative purity, are in fact different fractions of the whole.

The original zymoplastic substance used by Schmidt (1895) and his associates was an alcohol extract of tissue, and was thought to contain lecithin as the active factor. Morawitz (1905) showed that aqueous tissue solutions had the same effect and were, moreover, heat-labile; he concluded that the active factor was a protein, and called it "thrombokinase." Howell (1912) showed that both extraction by water or by lipoid solvents yielded active material, and he applied the now-popular term "thromboplastin" to the water-extractible fraction. He was able to show that the ether-soluble fraction was cephalin, which itself was active as a thromboplastin, though, when in combination with an unknown protein factor, the activity was greatly increased. The cephalin was heat-stable as regards activity, but the cephalin-protein complex was heat-labile. Confirmation for

the suggestion that cephalin was an active component of thromboplastin came from a number of investigators, including Gratia and Levene (1922), McLean (1916), and Quick (1936a), while lecithin was shown to be inactive (Clowes, 1917; Gratia and Levene, 1922). A particularly active cephalin preparation has been isolated from platelets by Chargaff and others (1936). Cohen and Chargaff (1940) supported Howell's belief that thrombokinase was a lipoprotein and suggested that the protein component was effective in orientating the cephalin at its surface. Chargaff and others (1944) have studied the chemistry of a lung preparation that is homogeneous in the ultra-centrifuge. They could not separate an active protein component. The lung preparations are more stable to heat than the brain extracts, and Lenggenhager (1936) has shown that even boiling does not greatly impair their activity. Quick (1942) has made similar observations.

Further indications that thromboplastin is a complex factor can be quoted. Though prothrombin can be activated by calcium and pure cephalin, the process is very slow compared with the reaction when crude tissue preparations are used (Seegers and others, 1938). A potent thromboplastin has been prepared from lung that contains no significant amount of cephalin, a finding of obvious importance (Charles and others, 1934).

It is possible that the action of Russell's viper venom may eventually throw some light on this problem. The fact that certain snake venoms were potent coagulants of blood was recognized by Martin in 1894, and their action was investigated by Lamb (1903) and Mellanby (1909). The prevalent use of oxalated or citrated blood as an indicator of activity, however, obscured the extreme potency of the venom of Russell's viper, which was pointed out by Macfarlane and Barnet (1934) in their search for a local haemostatic agent that might be effective in haemophilia. They found that haemophilic blood was rapidly clotted by extremely small amounts of venom, a dilution of one in many million still having a marked effect. The venom was incapable of clotting oxalated or citrated blood and had no effect on fibrinogen. It behaved in fact as if it were a thromboplastin.

Further work by Trevan and Macfarlane (1936) demonstrated the fact that the action of the venom was greatly increased by the presence of tissue lipoids, or lecithin, suggesting that the latter acted as a co-factor with the venom in its thromboplastic role. Macfarlane (1937, unpublished observations) found that the venom alone was unable to clot plasma from which the lipoid had been removed by extraction with petroleum ether, but that rapid coagulation occurred if the extracted lipoid or lecithin were mixed with the venom before addition to the plasma. He also observed that the coagulant extractable from human saliva behaved in the same way, since it would only clot the extracted plasma in the presence of added lipoid. These experiments extended the observations of Zak (1912) that plasma extracted with petroleum ether will not clot on recalcification unless the lipoid fraction is returned. Macfarlane and others (1941) obtained similar results with samples of plasma from which lipoid had been removed by high-speed centrifugalization, and suggested, as had Leathes and Mellanby (1939), that the venom was analogous to an enzyme (protein) component of natural thromboplastin, the lecithin being analogous to the natural lipoid co-factor.

The complex nature of thromboplastin has been emphasized by Feissly (1945a), who has extracted a thermostable phospholipoid from platelets and a thermolabile proteolipoid from plasma. Lenggenhager (1946) also considers that more than one factor is involved, since he supposes that prothrombokinin, a precursor of thrombokinin (? thromboplastin), is activated by proteolysis.

The findings of Zondek and others (1945) that thromboplastic substances derived from placental tissue were heat-stable when fresh but heat-labile after some days' storage emphasizes the difficulty of interpretation of published observations. Nevertheless it is clear that further experiments along the lines indicated may clarify the problem of the nature of thromboplastin, and recent work with haemophilic blood is already showing promising results.

Thrombin formation.—The nature of the reaction between thromboplastin, calcium, and prothrombin has given rise to endless discussion, fostered by uncertainty as to the "purity" of the various investigators' preparations of these factors, and the possibility that other undefined agents were involved.

The original view of Bordet and Delange (1912), supported in more modern times by Lenggenhager (1935), was that the three factors combined chemically to form a new compound, which was thrombin. It now seems most unlikely that this can be the case. It has already been said that the thrombin molecule appears to be smaller than the prothrombin from which it is derived, so that any simple combination of thromboplastin and prothrombin is, on the face of it, impossible. Moreover, it has been shown that thrombin contains no

demonstrable phosphorus (a considerable component of thromboplastin), and no calcium.

It would seem most likely, therefore, that thrombokinase activates prothrombin by removing some part of its molecule. Howell (1935) contended that this process consisted of the removal of heparin, which normally formed an inert compound with prothrombin. Quick (1936b), however, was not able to demonstrate the neutralization of heparin by thromboplastin, and, in any case, heparin exerts its main anticoagulant effect on the action of thrombin. There is, on the other hand, some evidence to favour the idea that one component of thromboplastin is an enzyme. The objection that thromboplastin has been stated to be heat-stable may well apply only to the lipoid co-factor which has been mistaken for the whole complex.

The significance of the activity of Russell's viper venom, which is almost certainly enzymatic, and its co-factor lecithin, has already been mentioned. There is additional support for proteolysis in the action of trypsin. Douglas and Colebrook (1916) observed that trypsin hastened the clotting of blood, a finding confirmed by Heard (1917), who found, moreover, that the enzyme was capable of coagulating oxalated blood. Mellanby (1935) and Mellanby and Pratt (1938), investigating this finding, came to the conclusion that trypsin activated the thromboplastin of the blood, and that the calcium present in the trypsin afforded sufficient to allow clotting. Eagle and Harris (1937), however, obtained the same result with calcium-free crystalline trypsin, and concluded that the prothrombin molecule was split proteolytically to form thrombin. Ferguson and Erickson (1939) concluded that the action of trypsin was to free a cephalin-calcium complex. It is of interest that a number of proteolytic snake venoms have a coagulant action similar to trypsin (Eagle, 1937).

It may be significant that substances with an anti-proteolytic effect have also been shown to be anticoagulant. The pancreatic trypsin inhibitor of Kunitz and Northrop (1936) is strongly anticoagulant (Ferguson, 1942), and the soya bean trypsin inhibitor isolated by Kunitz (1945) has been shown by Macfarlane and Pilling (1946b) and Macfarlane (1947) to act as an antithromboplastin.

The evidence seems, therefore, to favour the existence of a thromboplastic enzyme (kinase) which, in the presence of a lipoid activator and calcium, splits the prothrombin molecule. This enzyme is present with its activator in most tissues, and extracts of such tissues are spontaneously active. It is also probable that the enzyme exists in the plasma as an inert precursor which is activated by contact with a foreign surface or by some other factor resulting from such contact. This precursor is possibly identical with Lenggenhager's "proplasmakinin," Feissly's (1945b) "plasma thrombokinase," Macfarlane's (1945) "prokinase," and the so-called "antihaemophilic globulin" first described by Patek and Taylor (1937). Since the systems used by most investigators to test thromboplastic activity may be deficient in lipoid co-factor, but contain unsuspected and significant amounts of prokinase, it follows that the addition of lipoid may produce an acceleration of coagulation which may be taken to indicate the thromboplastic activity of the added lipoid.

An argument against the enzymatic, possibly proteolytic, activation of prothrombin is the apparent consumption of thrombokinase during thrombin formation. Though Eagle (1935a) considered that the amount of thrombin generated in a mixture of prothrombin, calcium, and thrombokinase was independent of the amount of thromboplastin, the work of Mertz and others (1939) suggests that, for low concentrations, a given amount of thromboplastin causes the conversion of a given amount of prothrombin, a finding taken to disprove the enzymatic nature of the reaction. Such an observation, however, might merely mean that it is the lipoid factor that is consumed during the process of activation.

The Inception of Blood Clotting

For many years the search for the prime mover of the coagulation mechanism has been undertaken without very convincing results. It has been recognized for more than a century that the introduction of tissue emulsions or extracts into the blood stream will result in intravascular coagulation in the living subject (de Blainville, 1834; Wooldridge, 1886). The coagulation of blood escaping from the body and mixed with the products of damaged tissue would seem, therefore, to be due to this admixture, and it has already been shown that such tissue fluids contain active thrombokinase. It can be argued, therefore, that active thomboplastin is capable of clotting blood without the intervention of any other agency; yet it is a familiar observation that blood removed with the greatest care to avoid admixture with tissue juice still clots in a short time on contact with glass or other foreign surfaces. The larger the surface area with which it is in contact, the more rapid the clotting, hence the coagulant effect of substances like cotton-wool or bandages and

their practical use in haemostasis, and the fact that the coagulation time of blood samples is faster in small tubes than large ones.

Lister (1863) was one of the first to point out the importance of such contact and showed that certain surfaces such as rubber delayed blood clotting, as compared with others such as glass. It soon became established that certain neutral substances such as paraffin (Freund, 1888), collodion, lusteroid (Lozner and others, 1942), and most recently silicone (Jaques and others, 1946), which are water-repellent and are not wetted by blood, have very little stimulating effect on coagulation, so that blood collected without tissue fluid contamination will remain fluid for long periods in vessels made of, or coated with, these materials.

The most familiar explanation of these observations is that originally put forward by Morawitz (1905), who supposed that active thrombokinase was liberated by the platelets which rapidly disintegrated when blood was shed. Calcium is necessary for such disintegration (Cramer and Dringle, 1912), and it has been shown by Tait and Green (1926) and Lampert (1930) that the surfaces active in promoting coagulation are those which cause disintegration of platelets.

In favour of this hypothesis it has been established that platelets contain thromboplastin, and significant changes in their morphology have been observed by Nygaard (1941) at the beginning of the clotting process.

It seems, however, that this is not the whole explanation. It is known, for instance, that the clotting time of whole blood as measured by the method of Lee and White (1913) is considerably influenced by small changes in thromboplastin (Quick, 1942), yet in severe thrombocytopenic purpura, in which the platelets may be almost entirely absent from the blood, a significant increase in the clotting time of the whole blood does not usually occur. An important finding of Lozner and others (1942) is that the effect of foreign surfaces on blood coagulation is largely independent of the platelets, there being acceleration or delay in glass or lusteroid respectively of the clotting time of plasma previously deprived of platelets by centrifuging. These authors, therefore, favour the view, already put forward by a number of others from Mellanby (1909) onwards, that there exists an inactive precursor of thrombokinase in the plasma and that this is activated in some way by contact with a foreign surface.

Recent observations by Brinkhous (1947) and Quick (1947b), in relation to the clotting effect in haemophilia, may have an important bearing on the normal first stage of coagulation. With the use of silicone, plasma can be prepared free of platelets and without surface contact action. On transfer to a glass surface such plasma will not clot without the addition of platelets. Brinkhous (1947) infers the existence of a "thrombocytolysin" that disrupts the platelets on contact with a foreign surface with release of thromboplastin. Quick (1947b) postulates a factor released by platelets on contact that activates "thromboplastinogen." It might equally be argued, from what has already been said, that platelets on rupture supply the "lipoid factor" which reacts with the contact-activated "prokinase" or thrombokinase precursor.

The nature of such contact action is almost certainly physical. As Pickering (1928) writes, "Electrical changes produced by contact of blood with a surface which it wets seems sufficient for the inauguration of clotting. A wetted surface becomes electro-negative and the presence of a negative charge implies an equal positive charge in the liquid in contact with it . . . a change of electrical conditions is thus a feature of normal clotting." What other factors intervene between such contact and the activation of prokinase (or thromboplastinogen) are not known. It has already been stated that Lenggenhager considers that proteolysis plays a part, a view favoured in principle by Wöhlisch (1940) and Ferguson and others (1947). If this is so, the whole fibrinolytic system, which results in the activation of the proteolytic enzyme plasmin (see Macfarlane and Biggs, 1948), may be concerned with the inception of blood clotting.

The Maintenance of Normal Blood Fluidity

Many workers on the subject of blood coagulation have been so preoccupied with an explanation of the change from fluid to solid that their theories hardly explain why the blood is fluid in the body. Yet it is clearly as important to the individual that his blood should not clot in his vessels as that it should clot outside them.

Normally the blood has an inherent stability in the body. The main reason why intravascular coagulation does not occur is the unbroken continuity of the vascular endothelial surface, which is inactive as regards clotting. Nevertheless, it is not only on this absence of foreign contact that the body relies. Disease may alter the vascular endothelium, but no more than a local deposit of platelets and fibrin may form. Trauma may cause extensive damage to tissues and vessels, with inevitable absorption of thromboplastin and

thrombin, but only local thrombosis of the affected vessels occurs in the majority of cases. Though intravascular clotting can, as has been said, be produced by the injection of tissue extracts, relatively large amounts have to be given to produce it, and even thrombin given intravenously may have no apparent effect (Davis, 1911).

The classical theory does not explain this reluctance of the mass of circulating blood to clot in the presence of active coagulants. It is almost certainly due to the presence of anticoagulant factors, which, though demonstrable, have received comparatively little attention and which probably maintain a dynamic equilibrium with coagulant factors that are slowly produced in even normal circulating blood.

It was early recognized that thrombin rapidly disappeared from serum after coagulation was complete. Schmidt (1892), in studying this problem, paid particular attention to the fact that after such spontaneous inactivation the thrombin could be regenerated by the addition of acid or alkali. Morawitz (1924) came to the conclusion that thrombin was converted into an inactive form which he named "metathrombin," and Weymouth (1913) and Gasser (1917) concluded that there is a substance, or antithrombin, present in serum with which thrombin combines and by which it is therefore inactivated. No further progress was made until 1935, when Lenggenhager stated that this antithrombic factor was associated with the albumin fraction of the serum, a finding confirmed by Quick (1938b), who later called it "albumin X" (Quick, 1942) and showed that it had a lesser affinity for thrombin than had fibrinogen. Glazko and Ferguson (1940) have called it the "progressive antithrombin" to distinguish it from heparin and its co-factor, which they called the "immediate antithrombin." It has been studied by Astrup (1944), who calls it simply "antithrombin." Wilson (1944) believes that the thrombin is adsorbed directly by the albumin, but Wöhlisch and Köhler (1942) and Grüning (1943) find that the antithrombin activity of serum albumin is removed by extraction with chloroform or ether. The ether extract is itself antithrombic and appears to be a lipoid. Antithrombin has, therefore, similarities to the serum antitrypsin, or antiplasmin, which is also a labile factor associated with serum albumin and extractable with lipoid solvents (see Macfarlane and Biggs, 1948), and it is of interest that proteolytic enzymes such as trypsin are capable of regenerating from a thrombin–antithrombin mixture (Wöhlisch and Köhler, 1942).

An anticoagulant which has been more intensively investigated is heparin. Discovered by McLean (1916), it was thought by Howell (Howell and Holt, 1918) to be the antiprothrombin required by his theory. Mellanby (1934) and Quick (1936b), however, were able to show that the main action of heparin was against thrombin and not prothrombin or thromboplastin, though Brinkhous (1939) later showed that there was some inhibition of prothrombin conversion in the presence of heparin.

The action of heparin on thrombin, however, is not direct. Howell and Holt (1918) showed that it had little effect on thrombin and fibrinogen, and Quick (1936b) showed that this was due to the absence of a co-factor present in the albumin fraction of whole plasma without which heparin loses its effect. This co-factor has also been studied by Astrup (1944), who finds that it is more labile than the serum antithrombin, being destroyed by heating to 56° C., and that it disappears during the process of coagulation. The heparin co-factor complex has been called "immediate antithrombin" by Glazko and Ferguson (1940), and "thrombin inhibitor" by Astrup (1944), the co-factor being called "thrombin co-inhibitor."

Heparin was purified by Charles and Scott in 1933 and found to occur in liver, lung, muscle, heart, and blood. The chemistry of the substance was studied by Jorpes and Bergström (1937), who established the fact that it is a mucoitin polysulphuric acid ester. Hölmgren and Wilander (1937) showed that the basophil granules of tissue mast cells are probably composed of heparin, a supposition recently confirmed by Oliver and others (1947).

It is likely (Astrup, 1944) that heparin exerts its effect by virtue of the strongly charged sulphuric acid group. Bergström (1936) has synthetized sulphuric acid esters of different polysaccharides, and some, including those of chitin, cellulose, and pectin acid, have an anticoagulant action like that of heparin. It is significant that the anticoagulant azo dyes also contain a sulphuric acid group.

Jaques and others (1942) have shown that the heparins isolated from different animal species have different anticoagulant activity.

The importance of heparin as a physiological anticoagulant is still uncertain. If it is present in normal blood, it is in small amounts, but certain conditions as, for example, anaphylactic shock (Howell, 1925; Jaques and Waters, 1941) or heavy exposure to irradiation (Allen and Jacobson, 1947)

may cause such a rise in blood heparin that partial or complete incoagulability results.

Other anticoagulant agents in the normal clotting mechanism, though postulated, have not been definitely established. Collingwood and MacMahon (1912) have suggested an antithromboplastin, and Tocantins (1944a, b; 1945) has produced evidence that such a factor may exist, being, he thinks, increased in amount in haemophilia.

Quantitative Studies on the Coagulation Factors

It is not until the study of a particular reaction has reached the stage at which the factors concerned can be measured quantitatively that it can be termed a scientific investigation. Though certain of the more stable clotting factors have been the subjects of quantitative study, it is only in the past few years that the main developments along this line have taken place. The stimulus has undoubtedly been the discovery of vitamin K and the condition of hypoprothrombinaemia, both being dependent on the determination of prothrombin concentration; and the use of anticoagulants in the control of thrombosis must itself be controlled if the treatment is not to be more dangerous than the disease. It is obvious that a general change in attitude towards the problem of coagulation has sprung from these necessities, and its study from being a pleasant pastime for argumentative academicians has grown up overnight into a practical science with vital applications.

The effect of the increased precision of thought and technique which this new development has demanded has not, however, been to simplify the general picture of the coagulation process. On the contrary, it has been found that new factors and new and complicated interrelationships must be postulated if the new quantitative results are to be intelligible. These have, for the moment, been wedged into the already rather rickety framework of the "classical theory." Whether it will continue to bear this increased weight, or whether it will collapse to be replaced by a new structure, remains to be seen. These forebodings can be illustrated best by considering the various quantitative procedures and the complications that have arisen from them.

Simple blood coagulation time.—The time taken for blood to clot after its removal from the body provides the oldest and simplest of the quantitative approaches to the problem of coagulation and was estimated by most of the early workers on the subject. Many of them devised their own methods, some of considerable ingenuity, and it would be impossible to embark on even a brief survey of the instruments constructed, varying in complexity as they do from an inch or so of capillary glass tubing to a room full of electrical machinery.

In general the simplest methods are the best for ordinary purposes, and the methods in common use to-day require no special apparatus. Blood obtained by venepuncture is preferable to that from a skin puncture, because in the former case, assuming reasonable technical skill, there is a minimum admixture with tissue juice, which in capillary blood may be present in variable amounts, thus adding an uncontrollable variable to the estimation. For venous blood samples the method of Lee and White (1913) is usually employed. In patients in whom venepuncture is contraindicated capillary blood samples are usually tested by the methods of Dale and Laidlaw (1911), Wright (1893), Sàbràzes (1904), or Bürker (1907).

Prolongation of the simple coagulation time gives evidence of gross impairment of the coagulability of the blood, such as is met with in haemophilia, fibrinopenia, or severe hypoprothrombinaemia. It does not, however, give a significant indication of the level of fibrinogen until this is almost completely absent (Schmitz, 1933), prothrombin until it is less than 10 per cent of the normal value (Quick, 1945a), and calcium unless this is below the level at which tetany usually occurs (personal observation). In the majority of cases the main factor controlling the clotting time of whole blood seems, therefore, to be the concentration of thromboplastin (Quick, 1942). All other factors are normally present in considerable excess for the production of fibrin within the time of this estimation.

As such estimations are commonly performed there are a number of factors which may influence the results. Small amounts of tissue juice may greatly shorten the clotting time of haemophilic blood. Dirty tubes or syringes may introduce significant variations. Temperature must be controlled carefully, since it has a considerable effect. Fig. 3 illustrates the effect of varying temperature on the clotting time of normal blood in a mechanical coagulometer of the author's design (Macfarlane, 1938a). At the temperatures above 47° C. it was observed that clotting was poor, part of the fibrinogen being precipitated before coagulation could occur.

Fibrinogen and calcium.—The two substances which have been susceptible to ordinary biochemical assay methods are calcium and fibrinogen. The former has not involved any serious difficulty from the point of view of coagulation theory, though its exact part in the mechanism is still not known.

Fibrinogen.—The estimation of fibrinogen usually involves the clotting of a given amount of oxalated plasma by recalcification, removal of the fibrin, washing it free of soluble protein, and the determination of its mass by either drying and weighing or by nitrogen determination. Apart from technical errors and difficulties, this method assumes that all the available fibrinogen is converted into fibrin by this process, and

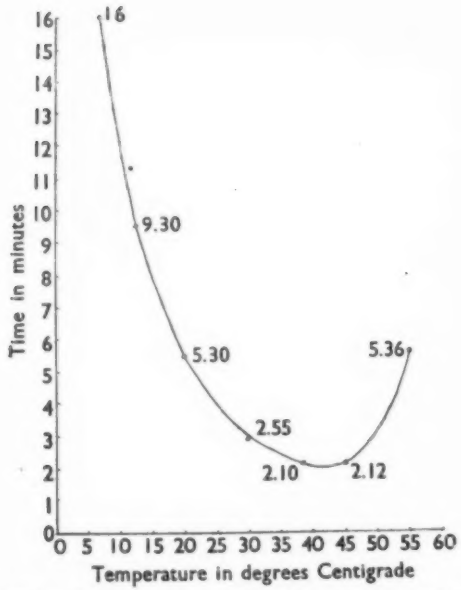


FIG. 3.—Curve showing the effect of temperature on the coagulation time of normal whole blood. (Macfarlane, 1938a.)

that one milligramme of fibrin is derived from one milligramme of fibrinogen. The latter assumption is probably correct, but the former is not necessarily so. In any condition in which thrombin production is defective only a part of the fibrinogen may be converted, and subsequent addition of thrombin may produce another clot. It is also doubtful if even added thrombin removes all the protein which should strictly be regarded as fibrinogen. It has been found in the writer's laboratory that after a given amount of fibrinogen has been clotted by thrombin there is still 10 per cent left in solution that can be precipitated by heating to 47° C. or by quarter-saturation with ammonium sulphate.

The estimation of thrombin activity.—The estimation of the activity of a given solution of thrombin is the essential basis of quantitative assay of any of the other coagulant factors, since, of all the reactions involved in clotting it is only the reaction between thrombin and fibrinogen that gives a visible effect. In principle, the thrombin to be tested is added to either oxalated plasma (Mellanby, 1933) or fibrinogen solution (Warner and others, 1936), and the clotting time determined. Mellanby (1933) defined a thrombin unit as that amount required to clot 1 ml. of oxalated plasma in 30 seconds at 37° C. For actual assay, Quick (1942) prepares a "standard thrombin" from a given volume of normal plasma, makes serial dilutions of this from full strength to 1/300, and adds 0.1 ml. of each dilution to 0.2 ml. of normal oxalated plasma at 37° C., taking the time of coagulation for each dilution. From the curve of clotting times plotted against dilutions, the strength of an unknown thrombin is read off by estimating the coagulation time with the same plasma. Such a method, of course, merely gives the value of the unknown in terms of the arbitary standard, and, as has been shown by Owren (1947a) and Astrup (1944), oxalated plasma is apt to give variable results even with the same thrombin dilutions. These difficulties are almost certainly due, in part, to the presence of antithrombin which will affect the longer clotting times of weak thrombin dilutions more than the shorter times (Fig. 4).

The use of fibrinogen solutions, prepared by removing prothrombin by adsorption with magnesium hydroxide and subsequent salt precipitation, allowed Warner and others (1936) to define a "thrombin unit" as that amount of thrombin that would clot 1 ml. of 0.08→0.1 per cent fibrinogen in 15 seconds at 28° C. There are, however, difficulties even with this refinement of the method. The reaction time is considerably influenced by such variables as *p*H, protein and salt concentration, and osmotic pressure (Quick, 1942; Owren, 1947a), and Astrup (1944) was forced to use a dried preparation of thrombin as a reference standard in his precise quantitative investigations. Some of these difficulties have been explained by Owren (1947a), who has shown that fibrinogen solutions prepared in the ordinary way

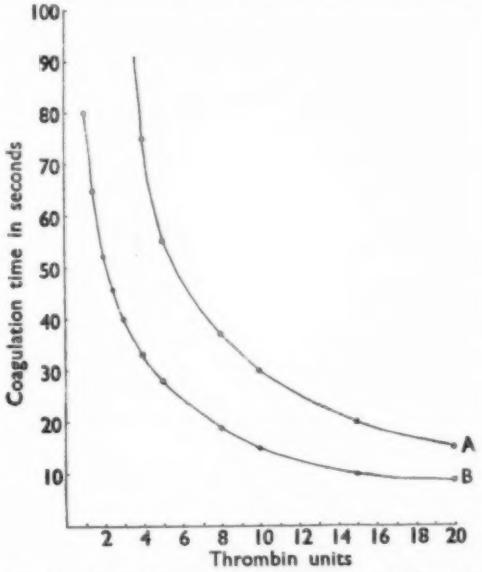


FIG. 4.—Curves showing the relationship of thrombin concentration to clotting time of oxalated plasma (*A*) and fibrinogen solution (*B*). (After Owren, 1947a.)

contain variable amounts of profibrin, which react more rapidly with thrombin than ordinary fibrinogen. Profibrin-free fibrinogen, which is therefore essential for accurate work, can be prepared by centrifuging off the bulk of the profibrin in the cold from freshly thawed frozen fibrinogen, and adsorbing the rest with kaolin. The resulting clear solutions of fibrinogen give regular results.

The estimation of thromboplastin.—The estimation of thromboplastic activity is one of great technical difficulty, since it requires adequate standardization and control of all the other known (and unknown) factors involved in the coagulation process. Fischer (1935a) used bird plasma as an indication of thrombokinase activity, but in view of the known species specificity of the clotting factors (Quick, 1942) such a system may not be applicable to mammalian thromboplastin. Mills (1921) deduced a formula for the assay of thromboplastin which has been also used by Astrup and Darling (1942). Astrup (1944), however, has found that there are differences in the qualitative reactivity of thromboplastins prepared from different organs which introduced uncertainty, brain thromboplastin being less reliable than lung preparations. Other fallacies have been discussed by Owren (1947a), who illustrates the inhibitory effect on coagulation of excessive concentrations of thromboplastin. Quick (1936a) and Aggeler and Lucia (1938) have used oxalated plasma for assay of thromboplastin.

The estimation of prothrombin.—The development of practical methods for the estimation of prothrombin was essential to the most fruitful research in blood coagulation, the discovery of vitamin K, the hypoprothrombinaemic states, and dicoumarin.

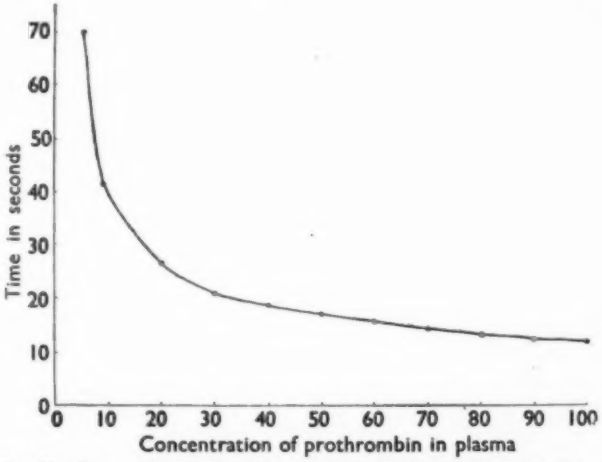


FIG. 5.—Curve showing the relationship of prothrombin concentration to coagulation time of recalcified oxalated plasma in the presence of optimum thromboplastin. (After Quick, 1942.)

Quick (1935b), during an investigation of the haemorrhagic tendency in jaundice, developed his method of prothrombin estimation which has proved so useful. In principle, it depends on the assumption that there are four reactants in the coagulation mechanism—thromboplastin, prothrombin, calcium, and fibrinogen. If any three of these are fixed, variations in the clotting time of the mixture must, it is assumed, be due to variations in the fourth factor. To determine the prothrombin concentration the concentration of thrombokinase, calcium, and fibrinogen should be controlled, preferably at their optimum concentration for coagulation, so that the clotting time of such a system should then be proportional to the concentration of prothrombin. In practice it is found (Quick, 1942) that fibrinogen variations in human plasma are not usually of such magnitude as to introduce a significant error, and the calcium concentration can be controlled by using oxalated plasma and a known amount of calcium chloride.

The remaining variable to be controlled is thromboplastin. In ordinary whole blood this is present in amounts so far below the optimum that variations in other factors have little effect on the clotting time. Quick therefore added "excess" thromboplastin—in the form of rabbit brain emulsion (later changed to acetone-extracted dried rabbit brain made up in saline (Quick, 1942)—to the oxalated plasma to be tested, together with the optimum amount of calcium chloride, and recorded the coagulation time. At first the results of this estimation were expressed in terms of the clotting time of the mixture, a normal control plasma being used. In 1938, however, Quick published a curve relating the clotted time by this method to the prothrombin concentration. This curve was constructed by making a number of dilutions of normal and abnormal plasma samples with saline, and from it percentage of prothrombin can be read off, assuming that 100 per cent is an average normal content, and that the thromboplastin used gives the clotting time with normal plasma that is equivalent to 100 per cent on the curve (Fig. 5). The theoretical basis of this curve is, of course, open to several objections. In the first place, as pointed out by Aggeler and others (1946) it was not constructed from a sufficiently large number of normal individuals, and the 100 per cent figure is quite arbitrary. More serious is the assumption that plasma diluted with saline is equivalent to undiluted plasma with a reduced prothrombin content. It is clear that dilution reduces all the clotting factors equally, including fibrinogen, while the naturally occurring hypoprothrombinaemia presumably affects only prothrombin. Many attempts

have been made to circumvent this difficulty, mainly by the use as a diluent of "prothrombin free" plasma, this being prepared by adsorbing normal plasma with Al(OH)$_3$ or by Seitz filtration. The results of such attempts to reproduce ranges of plasma dilution corresponding to natural prothrombin-deficient plasma have, however, been difficult to interpret. Quick (1945b) has emphasized the difference from saline-diluted plasma. Other workers (for example, Nitshe and others, 1947) have used fibrinogen solution as a diluent; and the whole complex problem has been discussed by Conley and Morse (1948) in relation to the reliability of thromboplastin preparations.

Many modifications of this so-called one-stage method have appeared. Smith and others (1939) introduced what is known as a "bedside" technique, which is carried out with whole blood and with much the same technique as that used for the Lee and White method, except that thromboplastin is added to the blood; and Kato (1940) has devised a micro-method also using whole blood. Fullerton (1940) suggested using Russell's viper venom as thromboplastin, the satisfactory results he reported having been confirmed by Page and Russell (1941). This material has the advantage of very high activity combined with almost complete stability when the venom is dried. On the supposition that the minimum clotting time indicates the optimum thromboplastin concentration, Witts and Hobson (1942) added lecithin to the venom, thereby so greatly increasing its activity that the normal prothrombin time was about 5 seconds. The use of Russell's viper venom, however, may give dangerously misleading results in patients receiving dicoumarin.

Several workers (Quick, 1942; Shapiro and others, 1942; Link, 1943; Allen and others, 1940; Aggeler and others, 1946) have recommended the practice of making one or more dilutions of the unknown plasma in order to obtain a longer clotting time which is more critically related to prothrombin concentration. It is true that the first part of the curve is flat and that little change in clotting time is observed until the prothrombin is 50 per cent or less, but the use of dilutions obviously introduces errors and fallacies of its own, as discussed by Fisher (1947).

Other complications appear when the prothrombin concentration is so low that long clotting times are obtained by this method. It has been found by Jaques and Dunlop (1945a, b) that the calcium concentration becomes very critical when low prothrombin concentrations are produced *in vivo* by the use of dicoumarol, or by adsorption of prothrombin by alumina *in vitro*, so that a slight divergence from the optimum has a considerable anticoagulant effect. Observations made by Dr. Rosemary Biggs (unpublished), extending those of Aggeler and Lucia (1938), show that thromboplastin concentration is also an increasingly critical factor as prothrombin concentration is reduced and that in such cases a slight excess of thromboplastin may actually inhibit clotting (Fig. 6).

Despite these theoretical and practical disadvantages Quick's original method provides a most valuable indication of the patient's liability to bleed, which is the main object of the majority of prothrombin estimations. The procedure has been standardized and its reliability investigated statistically by Aggeler and others (1946), but a few comments on the expression of the results might be added. In many laboratories it is the practice to express the results of a prothrombin

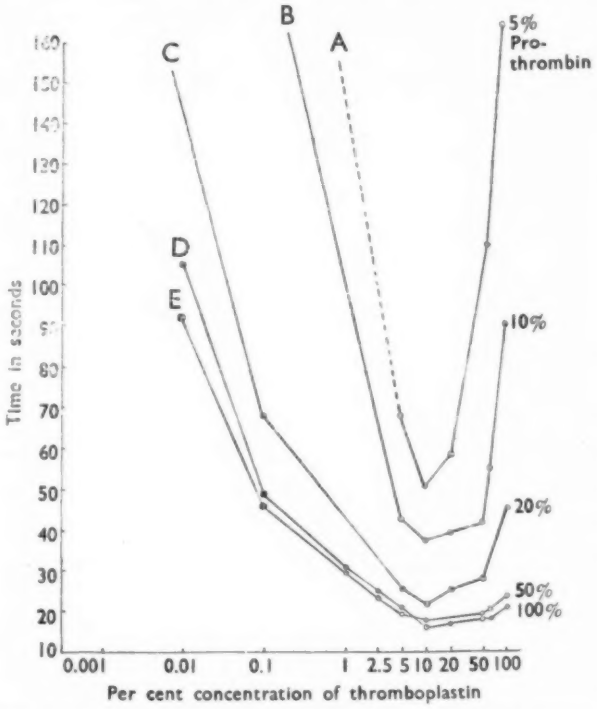


FIG. 6.—Curves showing the relationship of thromboplastin concentration to coagulation time for a number of different prothrombin concentrations ("100 per cent" thromboplastin = 1 g. acetone dried brain in 10 ml. saline). Curve A is for 5 per cent prothrombin, B for 10 per cent, C for 20 per cent, D for 50 per cent, and E for 100 per cent prothrombin. (Dr. Rosemary Biggs's data.)

determination as an "index" by means of the formula

$$\frac{\text{Normal Prothrombin Time} \times 100}{\text{Patient's Prothrombin Time}}$$

This, while convenient, gives a very misleading impression of the prothrombin concentration actually present in the patient's blood as calculated from Quick's curve. For instance, a "prothrombin index" of 33 per cent (i.e., patient's prothrombin time = 36 seconds; normal time = 12 seconds) is equivalent to 10 per cent prothrombin, and an index of 80 per cent is equivalent to 50 per cent prothrombin. Quick (1939) has suggested the use of the formula

$$\text{Prothrombin } \% = \frac{302}{\text{Prothrombin time} - 8.7}$$

assuming that the normal prothrombin time with the thromboplastin used is about 12 seconds. The use of this is preferable to the index, if the curve itself is not available. A difficulty in the use of both curve and formula is that of standardizing the thromboplastin to give a normal time of 12 seconds. The definition of the term "normal" in this respect obviously requires further investigation. In the writer's laboratory a number of curves have been constructed relating prothrombin concentration to clotting time for different levels of thromboplastin activity. By plotting the logarithm of the prothrombin concentration against the reciprocal of the clotting time, almost straight lines are obtained (Fig. 7). By the use of these, a given prothrombin time can be read off as concentration by interpolation, the activity of the thromboplastin used being known by testing against normal plasma. Rapoport (1947) has discussed other methods of calculating results.

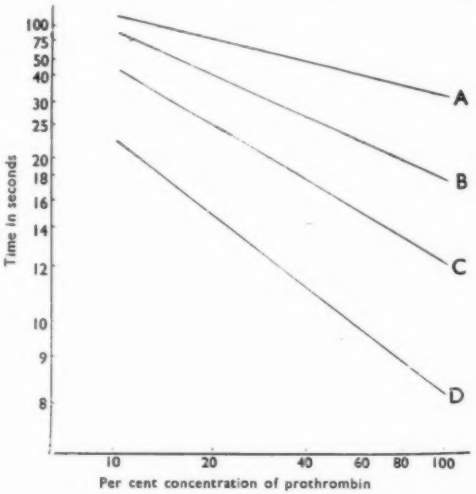


FIG. 7.—The relationship of clotting time to prothrombin concentration as it applies to Quick's method (determined by means of plasma dilutions) for 4 thromboplastin preparations of different potency, A, B, C, and D. Approximate linearity has been obtained by plotting the reciprocal of the clotting time (vertical axis) against the logarithm of the percentage prothrombin concentration (horizontal axis). (Dr. Rosemary Biggs's data.)

It has already been pointed out that Quick's method depends upon the assumption that the *amount* of prothrombin available controls the *rate* at which thrombin is formed, this rate in turn determining the clotting time of the plasma. In fact, only about 1 per cent of the prothrombin has been converted by the time that clotting occurs in normal plasma, full conversion, even in the presence of optimum thrombokinase, taking some minutes.

It would appear more scientific to use, instead of this dynamic method, dependent as it is on unknown factors controlling the rate of thrombin formation, a static method in which all the available prothrombin is converted into thrombin, which is then assayed by means of standard fibrinogen. Such a principle is the basis of the 2-stage method originated by Warner and others (1936). In this, the unknown plasma is first clotted by a small amount of thrombin (equivalent to about 1 thrombin unit), and the fibrin so formed is removed after 15 minutes when the added thrombin has been inactivated by the antithrombin of the plasma. The resulting fluid, containing all the original prothrombin but no fibrinogen, is then diluted to 1 in 20 with oxalated saline and activated with a mixture of buffered calcium chloride and thromboplastin. Samples of this reacting mixture in which thrombin is generated are then transferred to standard fibrinogen solutions and the clotting time of this is recorded. Dilutions of the reacting mixture are made to give a clotting time of about 15 seconds, this dilution therefore containing 1 unit of thrombin per unit volume as originally defined. The number of units per ml. of original plasma is then calculated from the final dilution of the plasma, and it is assumed that one unit of thrombin is derived from one unit of prothrombin.

The process of thrombin formation under these conditions in relation to time is illustrated in Fig. 8. It will be seen that the process starts slowly, and increases in rate until the thrombin concentration reaches a maximum which, instead of remaining constant, declines. The general shape of the initial part of the curve suggests an autocatalytic reaction (see Astrup, 1944), which is of considerable theoretical interest. The important practical points are the sharply defined maximum, necessitating repeated sampling during activation for its recognition and, even more important, the destruction of thrombin by antithrombin, indicated by the falling off of the curve. The authors of the method assumed that this antithrombin action was not significant at a dilution of 1 in 20 or over, but there is little doubt that it may be, and that it constitutes a serious drawback to a method already handicapped by the practical dis-

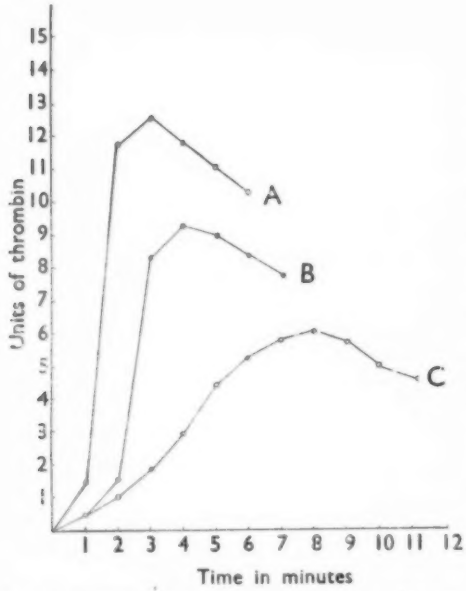


FIG. 8.—Curves illustrating the concentration of thrombin in a reacting mixture of thromboplastin, calcium, and diluted oxalated plasma, plotted against time, and for different dilutions of plasma. Curve A is for plasma diluted 1/25, curve B for plasma diluted 1/50, and curve C for plasma diluted 1/100. (Author's data.)

advantages of elaborate technique and reagents. Attempts have been made to overcome some of these disadvantages. The technical modification of Herbert (1940) provides a much simpler procedure, suitable for routine laboratory use. She makes an initial dilution of the test plasma, activates with thromboplastin and calcium, and takes sub-samples for thrombin assay, finding that the fine web of fibrin forming in the dilute reaction mixture does not interfere with the sampling. The thrombin units are read off by a calibration curve so that serial dilutions are unnecessary. Sternberger (1947) has also introduced a practical and possibly important modification by suppressing the antithrombin by the addition of alcohol. This, if successful, would eliminate the danger of loss of thrombin in slowly activating mixtures, but it has been found (personal observation) that the alcohol makes the end-point difficult to read, and it may have other unknown effects.

The 2-stage method has, of course, the academic advantage that it gives an estimate of prothrombin in absolute units and, when suitably modified, is not outside the scope of a pathological laboratory. It does not provide, however, the clinical information that the 1-stage test gives on the patient's liability to bleed.

Discrepancies Arising out of Prothrombin Estimation

The use of these two different principles for determining prothrombin on clinical and experimental material soon revealed a number of disturbing discrepancies in the results obtained. The first to appear was the relative normal prothrombin levels of different species. Quick found, for instance (1936b, 1941a), that rabbit plasma contained five times as much prothrombin as human blood, and cat blood about three times as much. Using the 2-stage technique, however, Warner and others (1939a, b) found human, cat, and rabbit plasma to have about the same prothrombin content of about 300 units per ml. It was also found by the 2-stage technique that stored blood maintained its prothrombin content for months (Warner and others, 1940) whereas by the Quick method it lost up to 90 per cent in the first week (Quick, 1943). Other discrepancies involving the prothrombin concentration in the newborn (Owen and others, 1939; Brinkhous and others, 1937; Quick and Grossman, 1939), and in jaundice, and also the level at which haemorrhage occurs (Brinkhous, 1940; Quick, 1938a) are listed in Table I.

TABLE I

DIFFERENCES BETWEEN RESULTS OF 1- AND 2-STAGE METHODS FOR ESTIMATING PROTHROMBIN

Source of prothrombin	1-stage	2-stage
Blood during storage	Rapid fall	Slow fall
Newborn babies	Sometimes normal	Consistently low
Obstructive jaundice	Low in some cases	Low in most cases
Cases beginning to bleed from prothrombin deficiency	10% prothrombin	30–40% prothrombin
Rabbit blood ..	500% of man	100% of man
Cat blood ..	300% of man	110% of man

It will be seen from this that the difference is not in the same direction in each case, the 2-stage giving lower results than the 1-stage in animals, jaundice cases, and babies, but higher on stored blood and at the haemorrhagic level. It is clear that the two methods are measuring different things, though both claim to measure prothrombin.

At first these discrepancies were considered to be due to differences in the "activity" or reaction rate of prothrombin (Warner and others, 1939b),

but later Quick (1943) made a fundamental observation relating to the discrepancy in the stored-blood estimation. He found that if a volume of plasma from which "prothrombin" had been removed by adsorption by alumina were mixed with a volume of stored plasma with a prothrombin time of 37 seconds (indicating 10 per cent prothrombin content), the prothrombin time of the mixture was 10 seconds, so that the mixture appeared to contain 100 per cent prothrombin and not the expected 5 per cent. Further work established the fact that plasma with a low "prothrombin" content following dicoumarol administration also had the effect of reducing the prothrombin time of stored plasma. From this and other evidence Quick concluded that "prothrombin" is made up of two components, a labile factor destroyed by slight degrees of heat, not absorbed by $Al(OH)_3$, and becoming inactive after a few days' storage (called at first component A), and a stable factor (component B) which retains its activity for long periods, is relatively heat-stable, is strongly adsorbed by $Al(OH)_3$, and is reduced by the *in vivo* effect of dicoumarol. In normal and dicoumarol plasma the labile factor is in excess.

Confirmation of the existence of such hitherto unrecognized factors required for thrombin production came rapidly and in some cases independently. Quick's observations were substantiated by Oneal and Lam (1945) and Munro and others (1945), but were disputed by Loomis and Seegers (1947), whose contribution will be discussed later. Seissly (1945c) and Fantl and Nance (1946a) obtained evidence of an accessory factor not included in the classical theory and which they concluded was required for the activation of prothrombin. Zondek and Finkelstein (1945) concluded that there was a thromboplastin co-factor which had been overlooked.

Meanwhile, Owren (1947a, b), working in enemy-occupied Norway and largely cut off from events in the United States, carried out an investigation which has brilliantly illuminated the problem of additional clotting factors. This work began in 1943, when a patient with a curious haemorrhagic diathesis came to his attention. She was a woman of 29, who from infancy had suffered from a severe haemorrhagic diathesis, clinically almost indistinguishable from haemophilia except for the absence of haemarthroses. There was no significant family history, no detectable physical abnormality not attributable to the effects of haemorrhage, and no abnormal blood findings apart from a long clotting time of 25 minutes. It was found, however, that, unlike true haemophilia, the prothrombin time by Quick's method was prolonged (70 seconds), indicating gross prothrombin deficiency. There was no reason to suspect vitamin-K deficiency, and the administration of vitamin K had no effect. Since all other clotting factors appeared to be normal and no anticoagulant could be demonstrated, the diagnosis appeared to be "idiopathic hypoprothrombinaemia." Many investigators would have been content (as were Rhoads and FitzHugh, 1941; Austin and Quastler, 1945) to accept this diagnosis and do no more. Owren, however, was more tenacious, and soon found that the addition of normal human plasma to the patient's plasma, even in as small a concentration as 10 per cent, would reduce the prothrombin time of the mixture to normal. Since this could not be explained by the simple addition of prothrombin, Owren tried the effect of adding normal plasma from which the prothrombin had been removed by adsorption with $Al(OH)_3$; 20 per cent of such plasma, itself incoagulable, reduced the prothrombin time of the patient's plasma to normal, thus demonstrating the presence of some factor that was not fibrinogen, since this was normal in the patient's plasma, nor prothrombin, nor calcium or thromboplastin, both of which had been ineffective in the prothrombin test. Owren called this factor which was deficient in his patient "factor 5," being the fifth clotting component. He went on to isolate factor 5 in a state of relative purity, and in an extensive series of experiments he showed that it is very labile, is not absorbed by $Al(OH)_3$, and is essential for the conversion of prothrombin to thrombin by thromboplastin and calcium. He showed that the injection of the factor into his patient reduced her coagulation time to normal. The failure by previous workers to recognize factor 5 seems to be due to the fact that, by ordinary methods of preparation, the fibrinogen and prothrombin fractions are heavily contaminated with it, so that its detection by virtue of its absence is difficult.

Quick (1947c) has not been slow to recognize Owren's factor 5 as being probably identical with his own "labile factor." Indeed, it seems probable that most of the discrepancies between the 1- and 2-stage methods are due to unsuspected variations in factor 5. The 1-stage method, being dependent as it is on the *rate* of conversion of prothrombin, is very sensitive to variations in the concentration of such an accelerator, while the 2-stage method, dependent upon the *amount* of thrombin generated, is much less so. This is one of the reasons why, for practical purposes, Quick's method gives a better indication of the patient's

liability to bleed. In the rare event of a factor-5 deficiency being suspected in a patient, factor 5 can be estimated by the method of Owren (1947a), which depends on the addition of dilutions of the test plasma to factor-5-free prothrombin, fibrinogen, thromboplastin, and calcium.

It seems to be established by weight of evidence, therefore, that what is usually regarded as prothrombin is a complex of two or more components. The objection of Loomis and Seegers (1947) was based on the fact that the prothrombin time of old plasma could be restored by the addition of fresh fibrinogen so that they considered Quick's labile factor to be a fibrinogen defect, but it is probable that their fibrinogen was contaminated with factor 5, which might thus be the active agent. The finding of Chak and Giri (1948) that the prothrombin time of stored plasma can likewise be restored by the passage of CO_2 suggests, however, the possible existence of a labile inhibitor that is developed during storage, and it is not, at the moment, clear in its implications.

Factor 5 or its equivalent has been accepted as an entity by some workers with such enthusiasm that they have discussed the priority of its discovery with considerable energy (*Lancet* correspondence, 1947). It must not be forgotten, however, that it is Nolf who has always maintained (Nolf, 1908, 1938, 1945) that five factors take part in coagulation. It is most probable that the factor called "thrombogen" by Nolf is identical with the newly described "factor 5" of Owren and "labile factor" of Quick. All three descriptions portray a relatively labile substance not adsorbed by such agents as $Al(OH)_3$, $Ca_3(PO_4)_2$, or Seitz filtration, and which is necessary for the coagulation, by thromboplastin and calcium, of a mixture of fibrinogen and the convertional "prothrombin." The credit for original recognition of this factor would thus appear to be due to Nolf.

Other factors.—Unfortunately factor 5 is not the only addition which is likely to be made to the coagulation theory. Owren (1947a) considers it necessary to postulate a derivative of factor 5 which autocatalytically activates prothrombin in the presence of thromboplastin and calcium. This is based on the observation that small subsamples of a reacting mixture of thromboplastin, prothrombin, factor 5, and calcium will clot oxalated plasma faster than it will clot an equivalent solution of fibrinogen, whereas a given amount of thrombin will clot the fibrinogen faster than the plasma. It is argued that something must be produced during the reaction that activates the prothrombin of the oxalated plasma, so that there is more available thrombin in this system than in the fibrinogen mixture. It can be shown that the thromboplastin and calcium are not active in this way in the absence of prothrombin. It is supposed, therefore, that a "factor 6" is generated that explains this autocatalytic reaction. These results are of interest, but their interpretation is not yet quite clear. Astrup (1944) obtained substantially similar results, and discussed the autocatalytic reaction at some length, being of the opinion that it is due to the activation of a plasma thrombokinase. Fischer (1935b) had made similar observations, and Bertrand and Quivy (1946)—using a photometric method for estimating clotting times—and Laki (1943) also support the necessity for postulating autocatalysis in the normal generation of thrombin, but further and carefully controlled experiments are required before the significance of this phenomenon is clear.

Quick (1947c), from studies of two families with inherited haemorrhagic diathesis associated with prolonged 1-stage prothrombin times, comes to the conclusion that in one of them the defect is due to a deficiency of prothrombin component B, already described. The other family, however, shows the deficiency of another prothrombin factor which is neither B nor the labile factor (previously called component A, very probably identical with factor 5). Quick, therefore, postulates the existence of a third prothrombin component deficient in this family, and proposed "to avoid confusion" by calling his original component A "labile factor," and the new component "component A." He suggests that it is the new component A which is reduced in vitamin-K deficiency.

Quick (1947a) has also—by means of an ingenious experiment involving the decalcification of plasma in silicone-treated tubes, by "amberlite"—shown that there is probably a co-factor required for calcium action in thrombin generation, this being independent of the other components of the prothrombin complex.

Abnormal Blood Coagulation

Artificial anticoagulants.—Of the anticoagulants used either *in vivo* or *in vitro* for therapeutic or experimental purposes, heparin has already been considered, since technically it is a naturally occurring anticoagulant. The various decalcifying agents such as citrates, oxalates, phosphates, and fluorides have been discussed in relation to the effect of calcium on the coagulation mechanism:

and the pancreatic and soya bean trypsin inhibitors have been considered from the point of view of their powerful and interesting antagonism to the action of thromboplastin.

An important group of anticoagulants are certain azo-dyes, including Chicago blue and chlorazol pink studied by Rous and others (1930), Huggett and Rowe (1933), and Huggett (1934), the latter worker considering that their action was on thrombokinase. Recent work by Astrup (1944) suggests that they are similar to heparin in their action, though they do not require the heparin co-inhibitor. Germanin, the anticoagulant action of which was noted by Mayer and Zeiss (1920), was found by Lenggenhager (1936) to act by stabilizing fibrinogen. Astrup (1944) states that this drug, and liquoid (sodium polyanethosulphonate), inhibits the thrombin-fibrinogen reaction possibly by reacting directly with fibrinogen. It has been observed by Fleming and Fish (1947) that penicillin has an anticoagulant effect, but its mode of action has not yet been investigated.

Hirudin, the anticoagulant secreted by the leech, was thought by Mellanby (1909) to be an antithrombin. This, and many other similar anticoagulant secretions produced by blood-sucking animals, have been largely neglected in modern times.

Dicoumarin.—The discovery of dicoumarin ranks, in the recent history of research on the haemorrhagic diatheses, second only in importance to the discovery of vitamin K. In 1929 and 1931 Roderick recorded that there appeared to be a prothrombin deficiency in animals suffering from toxic sweet-clover disease. Quick (1937) showed that the toxic clover hay produced a significant fall in the prothrombin level of animals to which it was fed, and, with the aid of prothrombin-time estimations as an assay method, Link and his coworkers (Campbell and others, 1941; Campbell and Link, 1941 ; Stahmann and others, 1941) were able to identify the toxic principle as 3,3′-methylene-bis-(4-hydroxycoumarin) and were then able to synthetize it in the laboratory.

Dicoumarin acts when given by mouth by reducing the prothrombin available for coagulation. There is a lag period after administration of from 24 to 48 hours before the reduction in prothrombin (Allen and others, 1942), suggesting an interference with production rather than a direct effect on existing prothrombin. Vitamin K given with dicoumarin prevents the fall in prothrombin (Shapiro and others, 1943a ; Davidson and others, 1945), but once the hypothrombinaemia is established vitamin K has little effect (Allen and others, 1942 ; Davidson and Macdonald, 1943 ; Meyer and others, 1942).

Quick (1947c) considers that dicoumarin causes a reduction of component B of prothrombin, but its mode of action is unknown. Its relation to the salicylates has been put forward by Lester (1944), and there are points of similarity between its structure and that of vitamin K which might suggest a possible competition in which the inactive dicoumarin displaces vitamin K (Fig. 2). A number of workers (Link and others, 1943 ; Meyer and Howard, 1943 ; Shapiro and others, 1943b ; Rapoport and others, 1943) have reported an effect similar to that of dicoumarin resulting from the prolonged administration of salicylates in man and animals.

Naturally occurring coagulation defects.—There is a group of haemorrhagic diatheses in which the primary cause of the abnormality is inefficient blood coagulation. This may be a result of an inborn, often hereditary, defect, or of an acquired deficiency of some necessary factor, or it may be secondary to a disease process. In general, the clinical manifestations of the different clotting defects are similar. The patient is liable to bleed for long periods of time from, or into, any tissue of the body in which the vessels are damaged by trauma or disease. In consequence, not only is there a danger of death from haemorrhage, but the effects of large effusions of blood into superficial or deep tissues, into joints, the nervous system, or internal organs, may be crippling or even fatal. There is thus a *generalized* haemorrhagic diathesis, in distinction to that of the purpuras, which is usually localized to the skin and mucous membranes (Macfarlane, 1941). As regards pathological investigations, the platelet count, the bleeding time, and the capillary resistance tests are typically normal, the coagulation defect being the only positive finding. Table II shows a classification of these conditions based on the coagulation defects thought to be responsible.

Haemophilia.—Many attempts have been made in the last 50 years to settle the problem of the slow coagulation of haemophilic blood, and to many of the investigators concerned it seemed that a solution had been found only to be refuted by others. The earliest conclusions, curiously, were nearer the most recent views than those that intervened. Sahli (1905) thought that thromboplastin was lacking in this condition, a view supported by the observation of Morawitz and Lossen (1908) that haemophilic blood clotted rapidly on

TABLE II

A CLASSIFICATION OF THE HAEMORRHAGIC DIATHESES DUE TO DEFECTIVE COAGULATION

Condition	Causation		Coagulation defect
Haemophilia	Hereditary		Thromboplastinogen deficiency
Pseudo-haemophilia	Idiopathic or secondary		
Hypoprothrombinaemia	Idiopathic or hereditary		Component A deficiency
	Vitamin-K deficiency:		
	1. Mal-absorption	Jaundice : sprue	
	2. Congenital	Neonatal	
	3. Mal-utilization	Liver disease	
	Hereditary: idiopathic		Component B deficiency
	Dicoumarin poisoning		
	Idiopathic		Factor 5 deficiency
Afibrinogenaemia	Hereditary		Fibrinogen deficiency
	Liver disease		

the addition of tissue extract, suggesting that the rest of the clotting mechanism was intact.

These observations were extended by Fonio (1914), who showed that normal platelets clotted haemophilic blood in a normal time. Attention was thus directed towards the platelet function in haemophilia, and it was suggested by Minot and Lee (1916) that they were functionally defective in this condition, a conclusion supported by Birch (1932).

It was soon pointed out, however, that these experiments, involving the transfer of normal platelets to haemophilic plasma and *vice versa*, might have other interpretations, and that a plasma factor contaminating the platelets might be the active agent (Eagle, 1935b; Patek and Stetson, 1936). The latter workers, and later Patek and Taylor (1936), showed that small amounts of platelet-free normal plasma were effective in clotting haemophilic blood, even in high dilutions and after filtration, thus explaining the familiar beneficial effect of transfusion of normal blood in haemophilia. Fractionation of the plasma showed that the active factor was associated with a globulin, a conclusion reached independently by Bendien and Van Creveld (1937). Considerable progress has been made along this line by a large group of workers in the United States, and it has been found that the factor in normal plasma effective to clot haemophilic blood is present in Cohn's Fraction I (Cohn, 1946), which contains also fibrinogen and some prothrombin. The factor is relatively heat-stable, so that fibrinogen and prothrombin can be destroyed without loss of activity (Lewis and others, 1946a). The administration of small quantities of this material to haemophilic subjects materially, if temporarily, reduces their clotting time (Minot and others, 1945; Lewis and others, 1946b) without inducing a refractory state sometimes seen as a result of repeated blood transfusions (Munro and Jones, 1943). It may therefore be assumed that in haemophilia there is a lack of a soluble plasma factor, associated with the globulin fraction, and probably related to, or identical with, the plasma thromboplastins already discussed. Reduced activity of this factor could explain the slow clotting of haemophilic blood which is due to the very small amount of prothrombin converted during the clotting process (Brinkhous, 1939), and the normal "prothrombin time" observed by Quick, Stanley-Brown, and Bancroft (1935) when thromboplastin is added.

The platelets, however, have once more assumed some indirect importance in the understanding of this problem. In 1941(b) Quick introduced a diagnostic test for haemophilia which depended on the

fact that centrifuging oxalated haemophilic blood increases the clotting time of the recalcified plasma to a much greater proportionate degree than does the similar treatment of normal blood. Though the exact implication of this reaction is not clear, it suggests that haemophilic plasma is abnormally dependent on the presence of platelets. This particular type of investigation has now been extended by Brinkhous (1947). Using platelet-free plasma samples obtained without any contact action by centrifuging in silicone-lined tubes, he made the following observations:

1. Neither normal nor haemophilic platelet-free plasma will clot on contact with glass, either separately or mixed together.
2. The addition of normal or haemophilic platelets to the haemophilic plasma did not result in clotting in glass.
3. The addition of *both* platelets (normal or haemophilic) and a small amount of normal plasma to the haemophilic plasma resulted in rapid coagulation in glass.

Brinkhous therefore concludes that both platelets and a plasma factor are essential for thromboplastic activity, and that the plasma factor, which he thinks is a "thrombocytolysin" that normally disrupts the platelets to furnish thromboplastin, is lacking in haemophilia. Quick (1947b) has made essentially similar observations by a similar technique, but concludes that the plasma factor is a thromboplastin-precursor (thromboplastinogen) normally activated by a platelet factor, the former being deficient in haemophilia. The writer has already suggested that the platelet factor may not be an activator but a lipoid co-factor.

Whether this defect is due to deficiency of "thromboplastinogen" or to the presence of an inhibitor is not yet clear. Tocantins (1943a, b; 1946) favours the presence of an "anticephalin" in excess. Feissly (1944) also finds an anticoagulant excess in haemophilic blood, associated with the albumin. On the other hand it is curious that there are persistent reports of some defect in the fibrinolytic system of haemophilic blood (Tagnon and others, 1942; Feissly, 1942; Macfarlane and Biggs, 1948), and the possibility that plasmin may be an activator of the thrombokinase complex cannot be ruled out. It may be mentioned that factor 5 is normal in haemophilia (Owren, 1947a).

Pseudohaemophilia.—The term pseudohaemophilia should be reserved to describe the states in which there is a clotting defect indistinguishable from that found in haemophilia, but which, for genetic reasons, cannot be regarded as the true condition. Joules and Macfarlane (1938) described such a diathesis acquired by a woman in late middle life. In no way could the clotting defect be shown to vary from that of true haemophilia, and apart from the history the clinical resemblance was complete. A similar case has been described by Maddison and Quick (1945). It has also been observed by the writer (unpublished) to occur as a complication of conditions with abnormal globulins, such as multiple myelomatosis, and as Hodgkin's disease. The use of the term pseudohaemophilia to describe cases of athrombocytopenic purpura, a condition which has no clinical, genetic, or haematological resemblance to haemophilia but which is still so described by recent writers (Estrer and others, 1946; Perkins, 1946), is to be deplored.

Hypoprothrombinaemia.—The historical background to the recognition of hypoprothrombinaemia and the existence of vitamin K has already been briefly outlined, and an admirable and detailed account of the conditions in which it occurs will be found in Quick's monograph (Quick, 1942).

The most important cause of hypoprothrombinaemia is vitamin-K deficiency, which may arise in several ways. In obstructive jaundice and biliary fistula the absence of bile salts reduces the absorption of the natural fat-soluble vitamin K from the intestine, with consequent lowering of the prothrombin content of the patient's blood and the development of the characteristic bleeding tendency that so often complicated this type of jaundice in the past. In haemorrhagic disease of the newborn there is, apparently, a congenital deficiency of vitamin K which can be prevented by the prophylactic use of the vitamin (see Lehmann, 1944). A less familiar cause of vitamin-K deficiency is the intestinal absorption abnormality associated with sprue, steatorrhoea, or chronic diarrhoea (Kark and others, 1940; Collins and Hoffmann, 1943). The haemorrhagic diathesis in such cases may occasionally develop rapidly and lead to diagnostic confusion. In liver disease with impaired function the prothrombin-deficiency is apparently due to failure to utilize vitamin K, and, unlike the other conditions mentioned, there is little response to its administration.

In animals, prothrombin deficiency may occur as a result of poisoning by feeding stuffs containing dicoumarin, a condition that has already been mentioned in relation to the discovery of the toxic factor. The now prevalent use of this material in human beings for therapeutic or prophylactic purposes is an important source of hypoprothrombinaemia, though, strictly, it cannot be called a "naturally occurring" condition. The effect of dicoumarin, being delayed and to some extent cumulative, is not always easy to control. In consequence there are many instances of the development of alarming haemorrhagic manifestations,

such as bleeding from operation sites, from mucous membranes, into the tissues, or from the kidneys (Allen and others, 1942; Crawford and Nassim, 1944; Shlevin and Lederer, 1944). Allen and others (1942) found that vitamin K did not reverse the effect of dicoumarin in such cases, though blood transfusion was more effective.

In controlling dicoumarin therapy, and in the detection of hypoprothrombinaemia in general, it is the writer's experience that Quick's prothrombin method performed with a brain thromboplastin gives clinically the most useful results. Prothrombin concentration is expressed as a percentage of normal by the use of a number of the curves already described. It is important to remember that with low concentrations of prothrombin such as are met with in dicoumarin poisoning, the concentration of calcium and thromboplastin becomes very critical, and that occasionally an excess of either may so delay the clotting that Quick's test has a longer time than the simple coagulation time of the same blood sample (Macfarlane, 1942; Fantl and Nance, 1947). Russell's viper venom has been found unreliable as a thromboplastin in assaying the effect of dicoumarin, since the prothrombin time may be considerably shorter than with brain extract and a dangerous overdosage of the drug may be given, an effect also observed by Lempert (1948). It must also be remembered that patients with a moderate hypoprothrombinaemia which is above the level at which bleeding occurs may be precipitated into a severe haemorrhagic diathesis by blood loss. The prothrombin concentration of the blood, already deficient, may be lowered disastrously by the dilution of the blood volume that follows severe bleeding, such as that occurring at operation or delivery.

The cases of so-called idiopathic hypoprothrombinaemia as described by Rhoads and FitzHugh (1941) and Austin and Quastler (1945) are probably examples of a deficiency of one of the newly recognized "prothrombin" components such as factor 5. Owren's (1947b) use of the term "parahaemophilia" for this condition seems to cause confusion, in particular since he has identified the precise deficiency in his own case. Quick's (1947a) two families with component-A and component-B deficiency respectively seem to be examples of inherited defects corresponding to those produced by vitamin-K deficiency, which apparently reduces component A, and dicoumarin poisoning which reduces component B.

Fibrinopenia.—Fibrinopenia may occur as a congenital absence of fibrinogen, possibly inherited as a recessive character (Macfarlane, 1937a) or secondarily to severe liver disease. Both are rare conditions, only 3 cases of the congenital form having been described in this country (Macfarlane, 1937a; Witts, 1942; Henderson and others, 1945). The subject has been recently reviewed by Yeager and others (1947).

When the absence of fibrinogen is complete, as was the case with the English patients, the blood is, of course, incoagulable by any means except the addition of fibrinogen. The rest of the clotting system appears to be intact, however (Macfarlane, 1937a), though thrombocytopenia may be associated with the disorder. It is curious that these patients are less severely incapacitated by their abnormality than is usually the case with haemophilia, despite the fact that any participation of blood coagulation in whatever mechanism prevents fatal bleeding is impossible.

The fibrinopenia secondary to some disease process is usually not complete so that diagnosis may be more difficult, since the clotting time is normal. The poor quality of the clots should be obvious, however, and a fibrinogen determination will reveal the defect.

Increased fibrinolysis.—It has been suggested (Reimann, 1941) that inherent fibrinolysis of the clots formed in certain cases may be the cause of a haemorrhagic diathesis. The evidence put forward is not convincing, and even in cases with active fibrinolysis no haemorrhagic diathesis has been observed by the writer that could be attributed directly to its action. Bacterial contamination, however, may activate plasmin to an extreme degree and produce considerable proteolysis that may be one cause of so-called secondary haemorrhage.

Circulating anticoagulants.—It has already been stated that anaphylactic shock will cause an outpouring of heparin in animals and possibly in man. A similar effect has been observed by Allen and Jacobson (1947) in animals exposed to irradiation, an observation of importance in view of the increasing risk of such exposure in modern life.

Naturally occurring anticoagulants in demonstrable form as a cause of bleeding are of extremely rare occurrence, though cases have been described by Lozner and others (1940) and Fantl and Nance (1946b).

Hypercoagulability of the Blood

1. Artificial coagulants.—It is understandable that in the past, when the specific deficiencies now recognized to be responsible for coagulation defects were not understood, recourse should have been had to many strange substances thought to promote coagulation. Comparatively few agents have in fact the effect of hastening true coagulation other than those already partaking in the natural process. In recognition of this, attempts were made to extract these natural coagulants, and many proprietary preparations were derived from platelets, leucocytes, brain, muscle, lung, glands, milk, and so on. Since these were often intended to be given parenterally it is perhaps fortunate that few of them possessed the powerful coagulant effect claimed for them, so that if no benefit was derived from their use little harm came either. It soon came to be recognized that in haemophilia,

to the treatment of which these efforts were mainly directed, only the transfusion of fresh normal blood, or the globulin fraction derived from it, is effective in lowering the clotting time.

The use of calcium for diminishing the risk of haemorrhage is still prevalent, however. It is apparently based on the misleading statement by Wright (1893) that calcium reduced the clotting time of haemophilic blood, and there seems to be little other evidence to support its use.

For local application, two natural products, thrombin and fibrin, have proved themselves to be useful. There are now several preparations of thrombin which are extremely active and will produce coagulation within a few seconds. One of these is derived from the interesting "rabbit-clotting globulin" described by Parfentjev (1941) and Taylor and others (1941), and subsequently produced commercially. It must be stressed, however, that no matter how powerful the coagulant its mere surface application to a bleeding point is useless for haemostatic purposes. The flow of blood merely washes the coagulant away to form ineffective clots at some point removed from the site of haemorrhage. It is essential in addition to control the bleeding by temporary pressure to give the mixture of blood and coagulant time to clot *in situ*.

The haemostatic efficiency of a blood clot is increased if it is reinforced by a dressing or light plug. The disadvantage of such foreign material is that it has eventually to be removed, a process often resulting in a recurrence of haemorrhage. The use of preformed fibrin in the form of sheets or "foam" is a considerable advantage in this respect, as it serves as a dressing that is naturally disposed of during healing (Macfarlane, 1943). Such fibrin preparations have now been greatly improved by American workers and have been used with success in a number of conditions (Bering, 1944; Ferry and Morrison, 1944; Woodhall, 1944). Other digestible haemostatic dressings of artificial nature, such as gelatine sponge (Light and Prentice, 1945) and soluble cellulose (Seegers and Doub, 1944), have also been reported to be efficient.

Of the artificial coagulants, some, such as citrate, egg albumin, peptone, pectin, gum arabic, and others, have been given parenterally. Citrate, paradoxically, seems to have some slight and transitory coagulant effect (Neuhof and Hirschfeld, 1922), but very large transfusions of citrated blood to exsanguinated patients have, in the writer's experience, been associated with depressed coagulation. An egg albumin preparation was developed by Timperley and others (1936) for the treatment of haemophilia, but their early results have not been substantiated.

Of artificial coagulants for local use, the venom of Russell's viper, physiologically resembling a thromboplastin, has been found effective (Macfarlane and Barnett, 1934; Macfarlane, 1935) with the same reservations that apply to the use of thrombin. Other snake venoms have a coagulant action (Barnett and Macfarlane, 1934), and Rosenfeld and Lenke (1935) have used that of the Australian tiger snake as a local haemostatic.

Many other substances, mainly used out of respect for tradition, have been employed as local haemostatics, and it would be laborious and out of place to consider them here. An account of many of them is given by Pickering (1928). Some, such as ferric chloride and turpentine, are not true coagulants but precipitants of plasma protein, and they have a dangerously necrotizing effect on the tissues, so that their use is absolutely contraindicated in haemophilic patients in whom healing must be encouraged by all possible means. The cautery has a similar effect and should be avoided in haemophilic patients for the same reason.

2. Intravascular coagulation.—The clotting of blood within damaged vessels and those immediately adjacent to a traumatized area is, of course, a physiological response to injury and a safeguard against blood loss. Coagulation in such vessels occurs because the local production of coagulant factors overwhelms the local concentration of anticoagulant factors. This process extends until the junction of the affected vessel with one carrying flowing blood is reached, when the clotting factors are so diluted as to be ineffective. Similarly, on isolated areas of damaged endothelium there will be deposition of platelets followed by fibrin to build up the familiar white thrombus, but so long as the blood flow is maintained massive intravascular coagulation will not occur.

The uncontrolled extension of this physiological process or its appearance in remote parts of the body is a familiar hazard of post-traumatic and post-operational states, and of certain disease processes. Such extensive thrombosis may restrict the blood supply of a limb, or of a vital tissue, as in mesenteric, coronary, or cerebral thrombosis, and detachment of a clot may cause pulmonary or other embolism.

Since it is true to say that many more patients die of thrombosis and its effects than of inefficient coagulation, it is clear that the problem is of greater social importance than the haemorrhagic diatheses and would seem to deserve greater attention in this review. However, this mysterious and

unpredictable occurrence apparently is not primarily due to an abnormality of the blood-clotting mechanism, but to the combination of tissue damage and circulatory stasis that may displace the clotting–anticlotting equilibrium in favour of coagulation.

A further cause of thrombosis is infection by certain organisms. It is well known that most pathogenic strains of *Staph. aureus*, for instance, produce a toxin capable of rapidly clotting human blood. Since it is equally effective if the blood is oxalated, it was assumed to act as a thrombin. Smith and Hale (1944) have investigated the action of this toxin and have shown that it requires the presence of a co-factor, at the moment unidentified, that exists in most, but not all, samples of human plasma.

Many of the snake venoms already mentioned are powerful blood coagulants, and the rapidly lethal effect in small animals and the local gangrene in man following bites by such snakes is probably related to intravascular coagulation (Macfarlane, 1937b).

So far, no abnormality of the blood has been established as a reliable indication of impending pathological thrombosis. It is known that trauma, as such, increases the clotting efficiency of the blood. It is well known, for instance, that the platelet count increases (Tocantins, 1938) and that there is a rise in fibrinogen and the sedimentation rate, and a fall in the coagulation time. These changes, however, are physiological rather than pathological, and no quantitative estimate of their magnitude seems to have been related to the incidence of thrombosis.

Platelets and thrombosis.—There are, however, certain lines along which progress is being made. The importance of the behaviour of the platelets in thrombus formation has been stressed since the time of Bizzozero (1882). Recently the "stickiness" of platelets, obviously an attribute that controls their tendency to adhere to foreign surfaces, has been studied quantitatively by Wright (1941, 1942, 1944). She has found that in conditions associated with an increased platelet count, such as parturition and surgical operation, there is an increase in the adhesiveness. This may be related to the fact that young platelets are more adhesive than old ones and that rapid production from the marrow increases the proportion of young platelets. She has also found that anticoagulants, such as heparin and chlorazol-fast pink, decrease the adhesion of platelets as measured by her method. A similar decrease of platelet adhesion following dicoumarin administration has been observed by Spooner and Meyer (1944). The nature of platelet adhesion is at present unknown. It was supposed by some authors (Lenggenhager, 1936; Wright, 1946) to be the result, rather than the cause, of coagulation, being due to a film of fibrin forming on the platelet surface. The demonstration of platelet adhesion by Pinniger and Prunty (1946) in the blood of a case of congenital total absence of fibrinogen appears to dispose of this explanation.

Other blood changes in thrombosis.—Hirschboeck and Coffey (1943) have utilized a clot retraction-time estimation which they claim gives results related to the danger of thrombophlebitis and pulmonary embolism, and which, subject to confirmation, are of interest.

Waugh and Ruddick (1944a, b) have made an interesting study of the anti-heparin effect they have observed in the blood of patients kept at rest in bed and in acute infection. They find that heparin has less anticoagulant effect on the blood of such patients *in vitro* than in normal blood, an observation that is worth extending.

The work of Lyons (1945a, b) on the existence of a more reactive fibrinogen (fibrinogen B) that may be increased in conditions associated with thrombosis appears to be of considerable promise and should be followed up without delay.

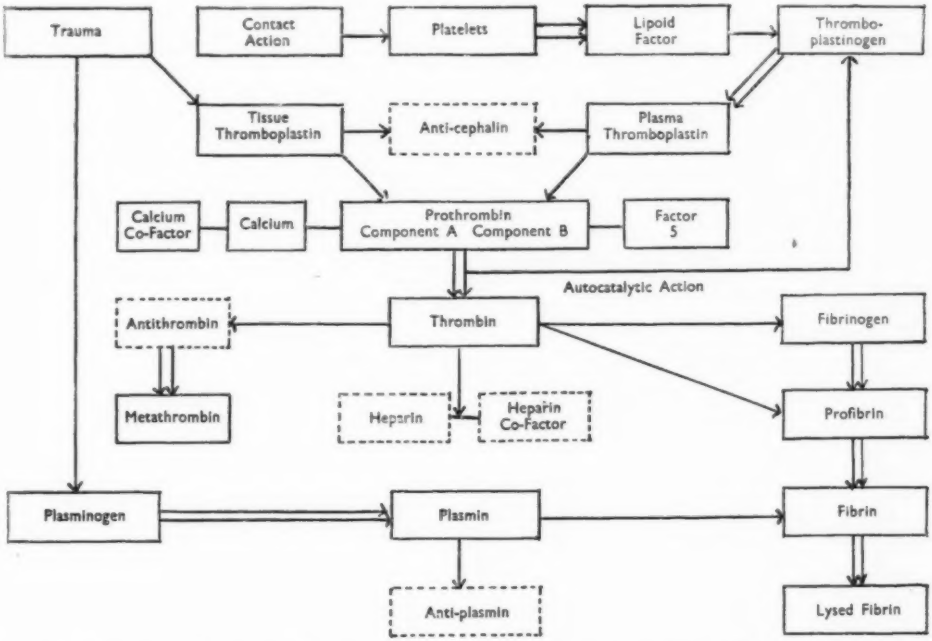
Finally, it may be said that there are many factors possibly related to the problem of thrombosis that have received very little attention. Fibrinolysis may be a process that normally limits the extension of intravascular clots, and it would be instructive to follow the development of the fibrinolytic reaction in relation to thrombosis. A systematic assay of the normal anticoagulant factors might also yield information of considerable value.

The prevention of thrombosis.—Once thrombosis is established very little can be done apart from attempts to prevent its extension. In this respect, and more particularly as a prophylactic measure in post-operative and post-partum cases, the use of the anticoagulants heparin and dicoumarin has been extensive and apparently successful. The pioneer work of Murray and others (1937), Best and others (1938), and Solandt and Best (1938) established that heparin was an agent preventing platelet agglutination and thrombus formation on damaged intimal surfaces, both active and non-toxic *in vivo*. Thereafter, heparin has been used on a large scale (Murray, 1940; Crafoord, 1939; Crafoord and Jorpes, 1941) as a therapeutic and prophylactic measure, and in vascular surgery. Heparin has a rapid effect which is of short duration, and in consequence it is usually advisable to give it by continuous intravenous drip (Quick, 1942) or by four, five, or more intravenous

injections daily (Crafoord, 1939), if the clotting time is to be maintained at a reasonably high level. This practical disadvantage might be overcome by combining heparin with some slowly absorbed base. Attempts in this direction have been made by Loewe and Rosenblatt (1944) and Evans and Boller (1946), but the local injection of such preparations has its own disadvantages. As regards dosage, this should be controlled by Lee and White clotting-time estimations, a time of about 20 or 30 minutes being desirable. "Units" of heparin are unfortunately still variable quantities, though it has been recommended by the Toronto workers (Best, 1940) that it should be fixed at 1/100 mg. of their pure barium salt of heparin. An initial dose of about 10,000 units is usual for an adult, subsequent doses being regulated by the patient's response.

Since ordinary preparations of heparin have a transitory effect, untoward haemorrhage can usually be controlled by merely stopping administration. Cerebral haemorrhage, however, is a real danger in patients with infective endocarditis treated with heparin (Witts, 1940).

Dicoumarin.—The difficulty of maintaining the effect of heparin, which is a relatively safe drug, has led to the use of the slowly acting, but more dangerous, dicoumarin. Introduced by Butt and others (1941) and Bingham and others (1941), it has had an extensive clinical application with apparently satisfactory results from the therapeutic point of view (Allen, 1947; Zilliacus, 1946). It must be mentioned that the incidence of thrombosis in the treated cases cannot be compared legitimately with the incidence of thrombosis in cases treated in the days before anticoagulant therapy was instituted, since other factors, such as avoidance of immobilization, reduction of infection, and other improvements may also have reduced the liability to thrombosis. Such comparisons, however, are made by the authors quoted. Not all authors agree, moreover, as to the therapeutic value of dicoumarin (Wasserman and Stats, 1943) or as to the theoretical basis for its use (Moses, 1945).



—A diagrammatic synthesis of the factors probably concerned in coagulation, and their interrelationship. A single arrow signifies "reacts with." A double arrow signifies "produces." A line joining two factors signifies "in conjunction with," more precise information in this case being not available. Anticoagulant factors are outlined with a dotted line.

As regards dosage, it is usual to begin with 5 mg./kilo body weight on the first day, followed by 1.5 mg./kilo daily (Quick, 1942) until the prothrombin concentration as measured by Quick's method is between 20 per cent and 30 per cent. Below 10 per cent there is a serious risk of bleeding, which once established may be difficult to stop even by large doses of vitamin K and blood transfusion. Control of the action of the drug is not always easy. There is a variable lag period, so that there may be a long delay before a significant fall in prothrombin, and continuing depression after cessation of administration, both complicated by variations in the susceptibility of individual patients. The technical difficulties of prothrombin determination in patients receiving the drug have already been noted.

Conclusion

From the rather confused collection of facts, observations, and opinions discussed in the foregoing review it is possible by a (perhaps unjustified) selection to build up a composite picture of the coagulation mechanism. This, unfortunately, is considerably more complicated than the older conceptions had visualized. It is illustrated diagrammatically in Fig. 9, which is a synthesis embodying those factors which seem reasonably well established. It will be seen that coagulation is initiated by admixture with tissue fluid, which provides active thromboplastin, or, what is probably more important, by contact with a foreign surface. This causes rupture of platelets with the consequent availability of some activating factor, or co-factor, which reacts with the thromboplastinogen (using Quick's term) to form active thromboplastin, which in turn reacts with the "prothrombin complex." This complex is, for the moment, shown as a combination of calcium, calcium co-factor, prothrombin components A and B, and factor 5. The exact relationship of these components to each other and to thrombin production is not clear at present, and a more specific designation is unjustified. The presence of all of them, however, is required for adequate production of thrombin under the influence of thromboplastin. Once formed, thrombin reacts with fibrinogen, possibly by the sulphydryl mechanism already discussed. It is possible that some intermediate fibrinogen product is formed, though whether this is "profibrin" or "fibrinogen B" or whether these two are identical is not yet established; the supposition that there are two different thrombins does not seem sufficiently supported to justify their inclusion. The so-called "autocatalytic reaction," illustrated in the diagram as the activation of thromboplastinogen by some effect of thrombin formation, is largely speculative and is merely one explanation of an observed phenomenon. Owren considers that an actual factor, "factor 6," is produced which activates prothrombin directly. The arguments for and against this contention are too involved to pursue here, but it can be said that since the activity ascribed to factor 6 might well be due to one of the other known factors involved, or even to a physical effect, and since the assumption of its existence complicates, rather than simplifies, the concept of thromboplastic action, the principle of Ockam's razor, never very popular with the constructors of coagulation theories, should reluctantly be applied.

The diagram illustrates also the probable inhibitors of coagulation, and it should be realized that only when the rate of production of the clotting factors exceeds the rate at which they are neutralized by inhibitors can coagulation occur. The fibrinolytic system is included, partly for the sake of completeness, since the disposal of blood clots is a physiological necessity, and partly as a reminder that it may play a much more important part in the coagulation mechanism than is now apparent.

It may seem curious that blood coagulation, which is after all a subsidiary and non-essential fragment of total bodily activity, should involve the multiplicity of factors illustrated, and a complexity which grows rather than resolves with increasing knowledge. Perhaps it is that in this particular subject the rather intensive investigation it has received has merely revealed more clearly the almost limitless complexity that underlies even the simplest of biological processes. It may be, however, that the conception of separate interacting factors is wrong in principle, being based on the artificial reactions of portions of plasma removed by force. Future workers may possibly agree with Pickering, who wrote twenty years ago that "instead of postulating a large number of bodies reacting in unknown ways, the plasma is seen as a unit capable of easily reacting to foreign substances by its unsatisfied side-chains, but returning to a normal condition when the foreign material has been eliminated."

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THE DETERMINATION OF PACKED CELL VOLUME FROM BLOOD AND PLASMA GRAVITIES IN INDIAN SOLDIERS

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Phillips and others (1945) recommended the use of standard copper sulphate solutions for determining the specific gravity of whole blood and of plasma. From these two values they calculated the blood cell volume using Ashworth and Tigertt's (1940–1) equation:

$$(1) \qquad CV = 100 \times \frac{Gb - Gp}{Gc - Gp}$$

where CV is the volume in ml. of packed cells in 100 ml. of blood, and Gb, Gp, and Gc are the specific gravities of the whole blood, plasma, and packed cells respectively. For Gc the normal value of 1.0970 was assumed.

The object of this investigation was to find out how far this method could be employed usefully in India Command in place of the usual haematocrit technique, which requires equipment not always available. Assessments of mean corpuscular volume based on an inspection of dry blood films or of the cells in the counting chamber cannot be expressed in figures, and it has thus been difficult to obtain information as to the improvement or deterioration in individual cases or to compare the blood state of different groups of population.

Seventy-five samples of venous blood were taken, the red cell concentration, the haemoglobin content, and the packed cell volume were measured, and the mean corpuscular volume and mean corpuscular haemoglobin concentration calculated. The specific gravities of the whole blood and plasma were estimated by the copper sulphate method and the packed cell volume was calculated according to equation (1). The subjects were Indian soldiers, most of whom were healthy. The cases of severe anaemia were hospital patients suffering from post-dysenteric debility.

Methods

Venous blood was withdrawn without stasis into syringes sterilized not less than twenty-four hours previously by hot liquid paraffin; 2.5 ml. was measured into a neutral glass tube marked at this volume and containing 2 mg. per ml. of a 3:2 mixture of ammonium and potassium oxalates. All estimations were done within three hours after the collection of the blood.

Haemoglobin.—A Sahli-Adams "Hemometer" was used with square, non-fading, plano-parallel double-glass standards. This apparatus was restandardized against bloods the haemoglobin content of which was determined by the method of King and others (1944), in which an alkaline haematin standard prepared from crystalline haemin is employed.

Red blood cells.—A Spencer's bright-line counting chamber with the improved Neubauer ruling was used. Each count was performed twice in two dilutions prepared in two different mercury calibrated pipettes.

Packed cell volume.—Wintrobe's "Hematocrit" tubes of 3 mm. bore were used. The samples were centrifuged for one hour in a Clay-Adams "Senior Model" centrifuge at 2,500 r.p.m.

TABLE I

DISTRIBUTION OF THE HAEMOGLOBIN AND MEAN CORPUSCULAR VOLUME LEVELS AMONGST THE 75 INDIAN SOLDIERS INVESTIGATED

Number of cases		Haemoglobin in g.%						
		Below 5	5–7.9	8–9.9	10–11.9	12–13.9	14–15.9	16 and above
		6	6	9	13	14	22	5
Mean corpuscular volume in cu.μ	Total number							
Below 80	4	1		2			1	
80–99	33	2	1	1	3	4	18	4
100–109	13	1	1		2	6	2	1
110–129	15	2		3	6	3	1	
130–160	10		4	3	2	1		

Specific gravity.—Drops of whole blood and plasma were delivered from a capillary pipette into standard copper sulphate solutions varying in specific gravity by 0.001. Values falling between those of two adjacent standard solutions were interpolated to the nearest 0.0002 specific gravity.* While the effect of temperature is the same on the gravity of copper sulphate solutions and of plasma, this has not yet been proved to apply to whole blood also, and as the laboratory temperature varied between 12 and 35° C. it was noted for each measurement.

Calculation of CV.—From the specific gravities of whole blood and plasma, CV was calculated using the above quoted equation. The added oxalates do not distribute themselves absolutely equally between cells and plasma, but this difference has only a small effect on the calculated results. The specific gravity values are given in Table II, and if wanted the corrections for oxalates according to Phillips and others (1945) can be introduced at any time. Gc was taken to be 1.0970.

Results

Table I shows the distribution of the haemoglobin and mean corpuscular volume levels amongst the seventy-five soldiers investigated.

If one classifies a blood haemoglobin content of above 14 g. per cent as normal, one between 12 and 13.9 g. per cent as showing a tendency towards anaemia, and one below 12 g. per cent as definitely anaemic, then 36 per cent of the subjects can be regarded as normal, 19 per cent as mildly anaemic, and 45 per cent as showing pronounced anaemia. Half the cases had a mean corpuscular volume of below 100 cu. μ as calculated from haematocrit values; of the other half, 33 per cent were mildly and 66 per cent severely macrocytic, having mean corpuscular volumes between 100 and 109 and between 110 and 160 cu. μ respectively.

Table II shows the CV values in all seventy-five cases and compares the results obtained by centrifuging and by calculating from Gb and Gp.

The haemoglobin content for each sample is given, and the table is subdivided into sections according to the mean corpuscular volume. It will be seen that the CV value calculated from the specific gravity is usually lower than the one obtained by measuring the packed cell volume by centrifuging. The Gc of 1.0970 used for the calculation was obviously too high for our samples. It has to be pointed out that the originators found Gc to be 1.0970 in normal heparinized bloods; it should therefore be expected to be higher rather than lower in our oxalated suspensions. Had a correction for oxalates been introduced the differences between the two CVs would be slightly greater still.

There is an association between the differences in the two CVs and the mean corpuscular volume. This difference is significant since $0.05 > P > 0.01$ when tested by analysis of variance. The calculated CV is on the average 5.50 less in the bloods with a mean corpuscular volume below 100 cu. μ; the difference is -4.52 when the mean corpuscular volume is between 100 and 109 cu. μ and only -3.16 in the samples with mean corpuscular volumes of 110 cu. μ and above.

* The specific gravity of blood or plasma is measured by dropping the fluid into copper sulphate solutions varying by 0.001 in gravity. For example, if a plasma drop falls in a solution of s.g. 1.024 and rises in one of s.g. 1.025, the gravity of the plasma is between 1.024 and 1.025. If it rises in the one with the same speed as it falls in the other the plasma gravity is taken to be 1.0245. If it rises more quickly in the 1.025 solution than it falls in the 1.024 solution, the gravity is between 1.024 and 1.0245. If it is stationary in the 1.024 bottle the gravity is 1.024, if it is almost stationary but falls very slowly it is taken to be 1.0242 and so on.

TABLE II

COMPARISON BETWEEN CV FOUND BY CENTRIFUGING AND CALCULATED FROM SPECIFIC GRAVITY VALUES OF BLOOD AND PLASMA

Haemoglobin in g.%	Temp. in ° C.	Gb	Gp	CV found by haematocrit	CV calculated from sp. gr.	Difference in % CV (sp. gr.) / CV (haematocrit)
Mean corpuscular volume below 80 cu. μ						
4.5	19.2	1.0380	1.0282	20.0	14.2	−29
8.0	24.9	1.0480	1.0306	32.9	26.3	−20
8.5	13.9	1.0432	1.0265	29.0	23.7	−18
14.4	14.0	1.0545	1.0260	48.0	40.3	−16
Mean corpuscular volume 80–99 cu. μ						
4.8	15.2	1.0358	1.0260	20.0	14.0	−30
4.8	34.4	1.0370	1.0262	14.6	15.0	+ 2
7.5	15.2	1.0480	1.0290	29.0	28.0	− 3
9.0	19.1	1.0446	1.0240	33.3	28.2	−15
10.4	19.5	1.0480	1.0270	38.0	30.0	−21
10.5	15.2	1.0488	1.0298	36.0	28.3	−21
11.0	14.0	1.0505	1.0300	38.0	30.6	−19
13.0	16.0	1.0530	1.0280	44.5	36.3	−18
13.5	18.0	1.0540	1.0270	43.0	38.5	−10
13.5	15.8	1.0540	1.0280	41.0	37.7	− 8
13.8	19.3	1.0533	1.0270	45.0	37.5	−17
14.0	16.0	1.0540	1.0288	45.8	37.0	−19
14.2	23.3	1.0565	1.0295	47.5	40.0	−16
14.2	23.9	1.0550	1.0268	46.0	40.1	−13
14.2	16.0	1.0541	1.0268	45.0	38.9	−14
14.2	16.0	1.0550	1.0268	44.0	40.2	− 9
14.2	15.8	1.0570	1.0290	42.0	41.3	− 2
14.6	15.8	1.0551	1.0285	48.0	38.9	−19
14.8	16.0	1.0553	1.0259	49.0	41.3	−16
15.0	16.0	1.0565	1.0273	48.0	41.9	−13
15.0	16.0	1.0557	1.0257	48.0	42.0	−12
15.0	14.5	1.0560	1.0268	49.0	41.7	−15
15.1	16.0	1.0563	1.0268	47.2	42.0	−11
15.1	16.0	1.0579	1.0282	49.0	43.2	−12
15.4	15.8	1.0565	1.0260	48.0	43.0	−10
15.4	16.0	1.0560	1.0268	48.0	41.7	−13
15.6	15.8	1.0570	1.0266	48.0	43.3	−10
15.6	16.0	1.0598	1.0276	50.0	46.4	− 7
15.8	16.0	1.0573	1.0268	51.0	43.5	−15
16.0	15.8	1.0588	1.0259	48.0	46.4	− 3
16.0	15.8	1.0603	1.0280	51.0	47.0	− 8
16.4	15.8	1.0580	1.0269	50.0	44.4	−11
16.4	15.8	1.0589	1.0257	48.0	46.6	− 3
Mean corpuscular volume 100–109 cu. μ						
3.7	14.1	1.0305	1.0230	11.5	10.1	−12
7.6	16.4	1.0412	1.0270	22.0	20.2	− 8
10.1	15.2	1.0489	1.0279	33.0	30.7	− 7
10.5	11.7	1.0488	1.0270	31.8	31.1	− 2
12.0	14.5	1.0522	1.0295	42.5	33.6	−21
12.0	14.5	1.0515	1.0300	41.0	32.1	−22
12.7	18.2	1.0539	1.0295	39.0	36.2	− 7
12.8	14.1	1.0530	1.0283	45.0	35.9	−20
13.3	18.2	1.0540	1.0290	40.0	36.8	− 8
13.8	15.5	1.0578	1.0308	41.5	40.7	− 2
14.0	16.1	1.0518	1.0250	44.9	37.2	−17
14.0	23.2	1.0530	1.0250	44.6	38.9	−13
16.5	35.3	1.0576	1.0270	49.0	43.5	−11

TABLE II—*continued*

Haemoglobin in g.%	Temp. in ° C.	Gb	Gp	CV found by haematocrit	CV calculated from sp. gr.	Difference in % CV (sp. gr.) / CV (haematocrit)
Mean corpuscular volume 110–129 cu. μ						
2.2	16.0	1.0298	1.0256	7.5	5.9	−21
4.8	15.4	1.0400	1.0300	15.5	14.9	− 4
9.0	15.9	1.0442	1.0255	24.0	26.2	+14
9.0	18.2	1.0472	1.0275	30.8	28.4	− 8
9.6	27.5	1.0460	1.0260	32.0	28.2	−12
10.1	15.9	1.0508	1.0300	32.5	31.0	− 5
10.2	14.1	1.0466	1.0249	36.0	30.1	−16
10.7	17.8	1.0475	1.0222	36.5	33.9	− 7
11.2	15.3	1.0515	1.0305	37.0	31.5	−15
11.5	13.4	1.0508	1.0270	35.5	34.0	− 7
11.7	23.1	1.0522	1.0310	37.0	32.1	−13
12.6	15.3	1.0515	1.0249	40.5	36.8	−12
13.0	15.0	1.0510	1.0260	38.5	35.3	− 8
13.1	15.0	1.0515	1.0280	39.5	34.5	−13
14.8	15.1	1.0552	1.0280	45.0	39.5	−12
Mean corpuscular volume above 130 cu. μ						
6.0	13.8	1.0370	1.0250	18.5	16.7	−10
6.4	16.5	1.0378	1.0230	20.0	20.0	± 0
7.0	12.8	1.0380	1.0220	22.0	21.4	− 3
7.4	23.4	1.0440	1.0288	23.5	22.3	− 5
9.2	23.9	1.0478	1.0278	28.6	28.9	+ 1
9.5	15.1	1.0478	1.0280	34.0	28.7	−16
9.8	14.5	1.0493	1.0283	35.0	30.6	−12
10.5	18.9	1.0490	1.0269	36.8	31.5	−14
11.4	14.5	1.0502	1.0270	40.0	33.1	−17
13.3	12.1	1.0540	1.0270	47.0	38.5	−18

There is in this series the usual association between high and low mean corpuscular volume and high and low mean corpuscular haemoglobin concentration. Therefore there is a tendency for the difference between the two CVs to rise the less haemoglobin the cells contain—that is, the more Gc becomes an expression of the gravity of the non-haemoglobin protein of the blood cells (see also Chart).

If the calculated CV values are multiplied with a factor of 1.1 more satisfactory agreement with the true CV is obtained.

In the Chart is shown the scatter of the mean corpuscular volume values based on CV haematocrit and on the CV (specific gravity) calculated according to the equation

$$(2) \qquad CV = 110 \times \frac{Gb - Gp}{1.0970 - Gp}$$

where Gb and Gp are again the specific gravity of the oxalated blood and plasma respectively. The values obtained from blood having a mean corpuscular haemoglobin concentration of 30 per cent and above are entered as ●, those of bloods showing a mean corpuscular haemoglobin concentration below 30 per cent are shown as ▲. The diagonal line represents ideal agreement and all values should be found on it if the method were perfect. It will be seen that while the values are about equally scattered above and below the line of ideal agreement, divergencies in either direction are considerable. The figure also demonstrates that even on using the corrected equation a low mean corpuscular haemoglobin concentration tends to place the calculated results below the true value.

Table III shows the ranges in which mean corpuscular volumes based on equation (2) fall when compared with the true values. Seventy-two out of seventy-five cases fall within the limit set out in Table III. The three exceptions are the first two samples in Group 2 of Table II and the first sample in Group 4. All three samples have a haemoglobin content of below 5 g. per cent.

TABLE III

THE RANGE WITHIN WHICH MEAN CORPUSCULAR VOLUMES FALL WHEN BASED ON CV VALUES CALCULATED FROM EQUATION (2)

Number of cases	True mean corpuscular volumes (derived from haematocrit CV) in cu. μ	Range of mean corpuscular volumes calculated from specific gravities	Number of exceptions
4	70– 79	60– 75	
33	80– 99	75–105	2
13	100–109	85–110	
15	110–129	105–140	1
10	130–160	120–175	

It will be seen that in our hands the method of calculating CV and mean corpuscular volume from specific gravity was not accurate enough to differentiate normocytosis from macrocytosis and microcytosis respectively. Whereas the difference between the Gc in our samples from that given by the originators may be compensated by introducing the factor 1.1 into the original equation, no single correction can reduce the wide margin within which calculated results are obtained on either side of the true values.

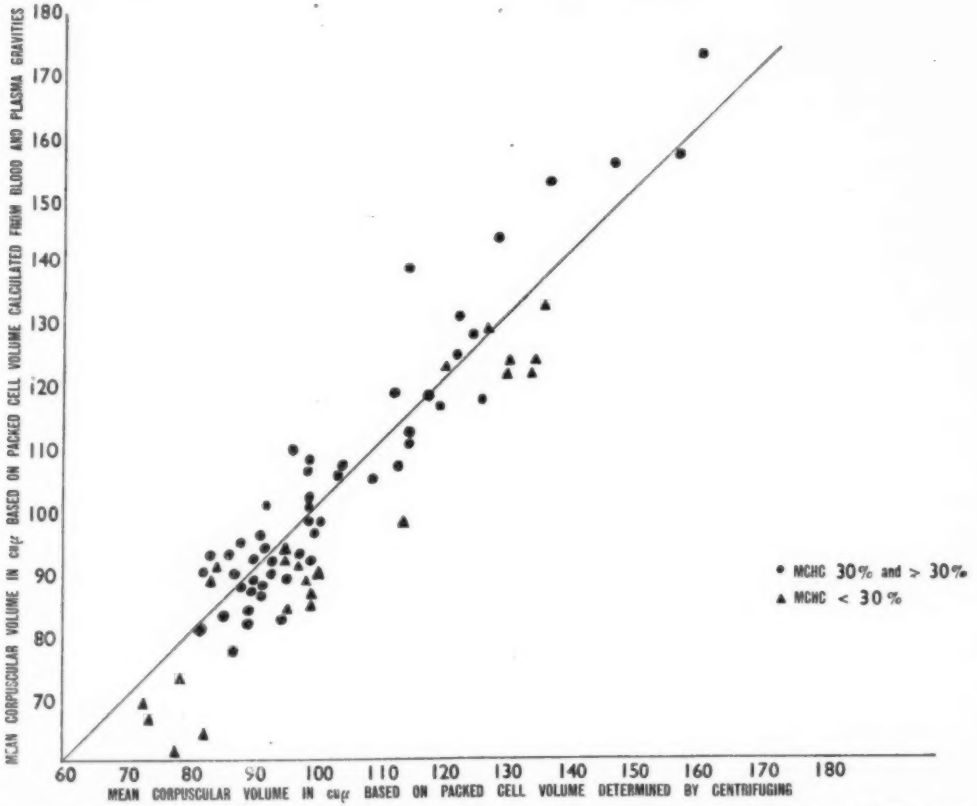

CHART.—The range within which agreement can be obtained between mean corpuscular volume derived from the haematocrit value and mean corpuscular volume based on a CV calculated from blood and plasma gravities. The influence of mean corpuscular haemoglobin concentration on this agreement is also shown.

Abscisse: Mean corpuscular volume in cu. μ derived from haematocrit values.

Ordinate: Mean corpuscular volume in cu. μ calculated from specific gravities.

● mean corpuscular haemoglobin concentration, 30 per cent and above.

▲ mean corpuscular haemoglobin concentration, below 30 per cent.

Summary

1. The haemoglobin content, the red cell concentration, the haematocrit value, the mean corpuscular volume, the mean corpuscular haemoglobin concentration, and the specific gravities of blood and plasma of seventy-five Indian soldiers were determined. Some of the subjects were hospital patients.

2. Nineteen per cent of the subjects were mildly, and 45 per cent more severely, anaemic. The mean corpuscular volume was below 100 cu. μ in one-half of the cases; of the other half, one-third showed slight and two-thirds considerable macrocytosis.

3. The CV values (ml. of packed cells in 100 ml. of blood) found by centrifuging (CV haematocrit) were compared with those calculated from blood and plasma gravities (CV specific gravity).

4. The CV (specific gravity) was generally 10 per cent lower than the CV (haematocrit). The per cent differences increased with smaller mean corpuscular volume and lower mean corpuscular haemoglobin concentration.

5. It is suggested that the differences between the haematocrit CV and CV determined from specific gravities were due to the cell gravities in the samples being below 1.0970, the value given for normal cell gravity by previous authors, and that these cell gravities were the lower the more they depended on the non-haemoglobin protein of the cells.

6. The difference between the haematocrit and gravity cell volumes could be fairly satisfactorily compensated by the introduction of the factor 1.1 into the equation from which CV specific gravity is calculated. However, mean corpuscular volumes based on CV specific gravity obtained in this way still diverged too widely on either side of the figures obtained from haematocrit values to make the calculation a useful method for clinical purposes.

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SUPPURATIVE MENINGITIS IN THE NEWBORN DUE TO COLIFORM BACILLI

BY

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Although infrequent, suppurative meningitis in the first month of life is now more commonly recognized than formerly. Barron (1918), reviewing meningitis in infancy nearly thirty years ago, found only thirty-nine cases of the suppurative form reported in children under three months, nineteen of which were in the newborn. From this he concluded that suppurative meningitis is a rare disease at this age.

Cruickshank (1930), in a general survey of neonatal deaths, found that meningitis was responsible in thirty-three out of eight hundred cases, about 4 per cent. Flensborg (1942) was able to collect 140 reports from the literature of suppurative meningitis in infants under one month old. In his own series of 374 necropsies on newborn infants, he found three cases (0.8 per cent) of suppurative meningitis. Still more recently MacGregor (1946) has recorded the pathological findings in 618 cases of neonatal deaths. Infections of all kinds were responsible for 30.7 per cent of all deaths, and for 65.5 per cent of the 241 deaths which took place after the third day of life. In a personal communication (1947), she states that there were eight cases of suppurative meningitis during the five-year period of her review (1939–43), a percentage of 1.3 of the total deaths.

Six further cases of suppurative meningitis, all of them caused by coliform bacilli, are reported below.

Case Histories

Case 1

The mother was a 10-para, aged 40 years. Labour was induced by rupture of the membranes on Oct. 18, 1946, and pains began early on the next day. She had no previous history of any infection of the birth canal or urinary tract. The patient was distressed during the first stage, and after twenty-one hours her pulse rate was 140 per minute and temperature 104° F. Foetal heart rate at this time was 150 per minute. The pyrexia subsided within three hours and did not recur. The child was delivered after a first and second stage lasting thirty-eight hours. The infant was a male and weighed 7 lb. 14 oz. At that time it was cyanotic for a few minutes only. Following this nothing abnormal was noticed until he was put to the breast for the first time after birth, when 7 hours old. He would not suck, and there was a sudden onset of nystagmus and some twitching of the limbs. The child remained restless, vomited several times, and 13 hours after birth had the first of numerous convulsions. Death occurred 16 hours 40 minutes after delivery.

Post-mortem examination.—The body was that of a full-term child showing early evidence of dehydration. There were no external marks.

Respiratory system.—There was some mucus in the upper air passages. Sub-pleural petechial haemorrhages were seen over both lungs, and on section the organs themselves were atelectatic, showing only small areas of expansion.

Central nervous system.—The skull was normal. The dura did not bulge, and there was no tear of tentorium or falx. In the subarachnoid space there was a considerable amount of thick, greenish pus, particularly over the base of the brain, from where it tracked along the superficial cerebral veins to the superior surface of the hemispheres. There was no excess of cerebrospinal fluid and no internal hydrocephalus. On section the cerebral hemispheres, midbrain, cerebellum, and medulla were normal. Basal arteries, mastoid air-cells, and venous sinuses were normal. Examination of cardiovascular, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormality.

Bacteriology.—Films of pus from the subarachnoid space showed many polymorphs and Gram-negative bacilli, some of which were intracellular. Cultures yielded a heavy growth of motile coliforms, giving the biochemical reactions of *Bact. coli communis.*

Histology.—The lung showed severe atelectasis of foetal type.

Various sections of the brain show acute purulent meningitis, with the majority of the cellular exudate made up of lymphocytes and other mononuclear cells, the number of polymorphs being small. Gram films show many Gram-negative bacilli in the exudate.

Case 2

The mother was a 2-para, labour being surgically induced on Dec. 16, 1946, as it was several weeks overdue. The labour was normal, and the child, a male, was delivered on Dec. 18. His weight was 6 lb. 12 oz., and for the first few days his condition was satisfactory, although he did not feed well and was of a poor colour. On the sixth day there was a sudden increase of temperature to 102° F. and marked increase of respirations. On the following day he developed twitching movements of limbs, on several occasions amounting to convulsions. Neck rigidity was present, and Kernig's sign positive. A diagnosis of meningitis was made, but before any further steps could be taken the child lapsed into coma, dying on Dec. 26, 1946.

Post-mortem examination.—The body was that of a well developed male infant showing slight cyanosis but no other external abnormalities.

Central nervous system.—The skull was normal. The dura was normal, with no tears of falx or tentorium. The brain showed severe flattening of the convolutions, and a diffuse purulent exudate was present in the subarachnoid space. At the base of the brain this exudate was yellow and opaque. The basal arteries, venous sinuses, and mastoid air-cells were normal. Cerebral hemispheres, mid-brain, cerebellum, and medulla were normal on section. Apart from some patchy atelectasis of both lungs, examination of respiratory, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormalities.

Histology.—Section of the brain showed a purulent exudate in the subarachnoid space of similar cytology to that in Case 1, with predominance of lymphocytes (Plate I*a*). Gram film of this section shows numerous Gram-negative bacilli, many of them intracellular and morphologically similar to *Bact. coli.* Unfortunately, no cultures were made from this specimen, but the finding of Gram-negative bacilli morphologically similar to *Bact. coli* and unaccompanied by any other bacteria suggests that they are the responsible organisms and that the condition is a coliform meningitis.

Case 3

Mrs. P., a multipara, was delivered at her home of a full-term male child on Nov. 4, 1946, the birth weight being 6 lb. 2 oz. The antenatal period and labour had been normal. The infant was breast-fed for two days, but sucked badly and was put on to artificial feeds. The child was still feeding very poorly on Nov. 10, and as it appeared to be getting weaker was admitted to hospital. On examination he was drowsy and cold; there was some cyanosis and signs of early dehydration. He vomited soon after admission. On Nov. 12 no abnormalities could be detected on examination, except for a possible weakness of the right arm, and he was still not feeding well. On Nov. 14 he was feeding better, but the skin was dry and inelastic, suggesting dehydration, but the fontanelle was not depressed. On Nov. 16 the pulse rate dropped from 150 on admission to below 100 per minute. Temperature still remained subnormal and the fontanelle tension was normal although general signs of dehydration were marked. There was, however, some separation of the cranial sutures. No neck rigidity was detected. On Nov. 17, 1946, the child was moribund and refusing all feeds. The fontanelle pressure was now increased, but there was still no neck rigidity. The pulse rate dropped to 64 per minute and death occurred on Nov. 18, 1946.

Post-mortem examination.—The body was that of a dehydrated male infant.

Respiratory system.—The upper air passages showed acute inflammatory changes, and the pleural cavities contained 15 c.cm. of straw-coloured effusion. On section the bases of both lungs showed a patchy pneumonic consolidation, and there were some areas of lung which had not fully expanded.

Central nervous system.—The skull was normal. The dura was bulging, and there was marked engorgement of the leptomeninges, with a thin sero-purulent exudate in the subarachnoid space, particularly marked at the base of the brain. The venous sinuses, basal arteries, and mastoid air-cells were normal. The brain was oedematous on section, but otherwise normal throughout.

Examination of cardiovascular, alimentary, hepatic, genito-urinary, and endocrine systems revealed no abnormality.

Bacteriology.—Films made from the subarachnoid space at necropsy showed numerous Gram-negative bacilli and pus cells. Cultures yielded heavy growths of coliforms giving the biochemical reactions of *Bact. coli communis.*

Histology.—Sections of the brain showed an acute purulent leptomeningitis with a considerable amount of haemorrhage amongst the exudate, and a cytology predominantly lymphocytic in character.

Sections of lung showed acute bronchopneumonic congestion and some collapse.

Cases 4 and 5 are siblings and two of quadruplets. As the hospital was bombed soon after their death the clinical notes were destroyed, and the histories are, therefore, lacking in detail.

Case 4

L. was the first delivered of quadruplets born prematurely on June 3, 1944, with a birth weight of 3 lb. 9 oz. She appeared well until the seventh day, when there was a sudden rise of temperature to 102° F. with the development of gradual abdominal distension and jaundice during the succeeding days. Cerebral screaming attacks also occurred. The blood count showed a mild leucocytosis of 25,000 cells per c.mm. During the performance of the differential count, which was normal, a macrophage (Plate I*b*) containing what appeared to be ingested bacteria was seen;

this suggested the presence of septicaemia, as did the continued fever and gradually increasing jaundice before death on June 19, 1944, at the age of 16 days.

Post-mortem examination.—The body was that of a small and premature female infant. The skin and all internal structures were deeply jaundiced.

Central nervous system.—The skull and dura were normal. Acute inflammatory changes were present in the leptomeninges with thin yellowish pus over the vertex and superior aspect of the cerebellum. There was also exudate over most of the basal cisterns. The cerebrospinal fluid in the ventricles was frankly purulent. The basal arteries, venous sinuses, and mastoid air-cells were normal. Sections of the brain were normal throughout. Examination of all the other systems revealed no abnormality.

Bacteriology.—Swabs taken from the brain and ventricles at post mortem showed large numbers of pus cells and Gram-negative bacilli in the stained films. Cultures yielded a pure growth of motile coliforms.

Histology.—Sections of the cerebral cortex showed acute inflammatory changes, but unfortunately the meninges from this specimen were lost.

Sections of the liver showed exudation of bile pigment, congestion, and fatty change.

Case 5

D. was born prematurely on June 3, 1944, the second to be delivered with a birth weight of 3 lb. 13 oz. His condition was satisfactory for the first few days, but he began to feed poorly at about the fourth day and when six days old developed a sudden rise of temperature. A day or so later there was onset of cerebral screaming attacks with gross abdominal distension. Treatment was begun with sulphathiazole by mouth, but there was gradual deterioration until death on June 14, 1944, when the child was 11 days old.

Post-mortem examination.—The body was that of a small premature infant showing some abdominal distension and generalized jaundice of the skin and internal structures.

Central nervous system.—The skull and dura were normal. The subarachnoid space contained a purulent exudate that was particularly thick over the vertex, superior aspect of the cerebellum, and tips of the temporal lobes. The cerebrospinal fluid was cloudy but not frankly purulent. Venous sinuses and basal arteries were normal. The right mastoid air-cells contained a little pus, but those on the left were normal. Examination of the cardiovascular, respiratory, alimentary, hepatic, genito-urinary, and endocrine systems revealed only scattered sub-serosal petechial haemorrhages.

Bacteriology.—Films from ventricular fluid, right mastoid, and subarachnoid space showed numerous pus cells and many Gram-negative bacilli. Cultures all yielded a heavy growth of *Bact. coli communis.* Culture from the left mastoid yielded only a few colonies of *Staph. albus.*

Histology.—There was acute purulent leptomeningitis of the brain, the underlying brain showing some congestion.

Sections of liver showed fatty change, exudation of bile pigment, and some areas of focal necrosis.

Case 6

The male infant was born by normal labour on April 29, 1947. The child appeared well until it was 3 days old, when it suddenly became grey, with difficult and distressed breathing. The birth weight was 8 lb. 3 oz., and at the onset of symptoms had fallen to 8 lb. 1 oz. Later the same day the temperature suddenly rose to 103° F. with grunting respiration. The infant had repeated atttacks of spasm of the hand muscles, similar to tetani. There was no neck rigidity, and the pulse was then uncountable. He was given oxygen and chloral, the temperature fell to 102.4° F. at 6 a.m. on May 3, but rose suddenly to 105.6° F. at 10 a.m. a quarter of an hour before death.

Post-mortem examination.—The body was that of a well-developed male infant. There was moderate cyanosis of mouth and finger tips, but no dehydration or jaundice.

Respiratory system.—The upper air passages contained a large quantity of purulent material and both lungs showed on section a generalized bronchopneumonia. The pleural surfaces were normal.

Central nervous system.—The skull was normal. The dura was normal, with no tear of falx or tentorium. A greenish exudate which had a faecal odour was present in the subarachnoid space. It covered the base of the brain and tracked to the vertex. There was no excess of fluid in the ventricular system. There were no flattening of the convolutions, and the brain was normal throughout on section. The basal arteries, venous sinuses, and mastoid air-cells were normal.

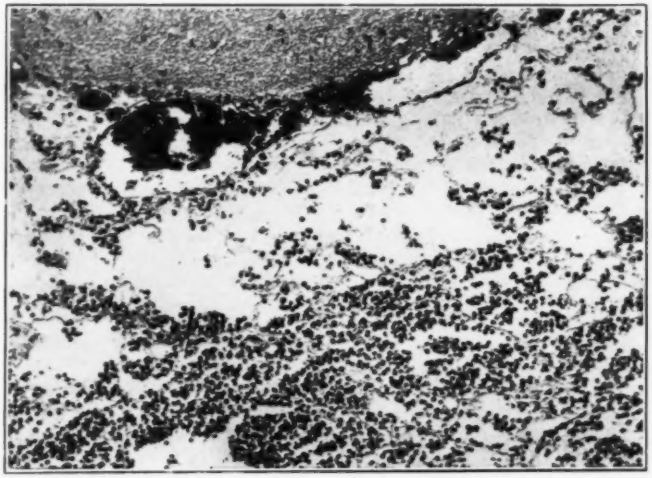
Bacteriology.—Swabs of the subarachnoid space showed numerous Gram-negative bacilli and pus cells. Cultures yielded a heavy and pure growth of *Bact. coli communis.* This organism was not inhibited by 75 units per ml. of penicillin but was by 100 units of penicillin. Swabs from lung showed numerous Gram-negative cocci and some Gram-positive bacilli. Cultures yielded a good growth of *Bact. coli communis* and a few colonies of *Staph. albus.* The coliform in this culture showed similar results to penicillin sensitivity tests as did that isolated from the brain.

Histology.—Sections of the brain showed a purulent leptomeningitis.

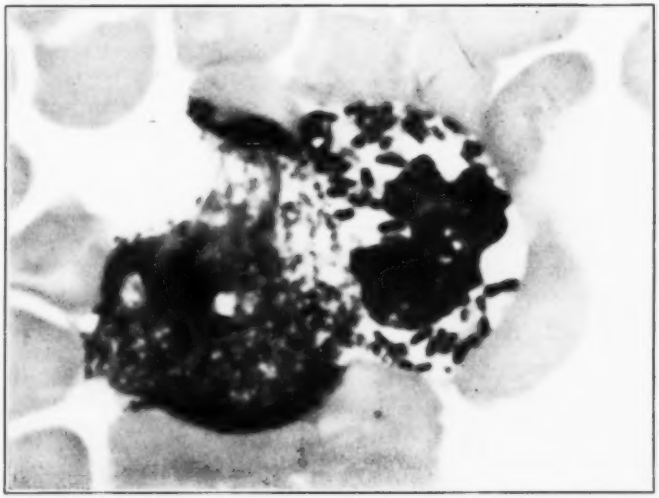
Discussion

Incidence.—The age at death in Cruickshank's (1930) series of suppurative meningitis cases varied from 2 to 17 days, 88 per cent of the infants dying between the fifth and thirteenth day.

In Levinson's (1945) group of cases there were eight under one month, and five of these were in infants 14 days old or less; the youngest was



(*a*).—Section of the brain in Case 2, showing a purulent exudate in the subarachnoid space, with predominance of lymphocytes.



(*b*).—Macrophage containing what appear to be ingested bacteria, Case 4.

PLATE I

days, and the eldest 21 days. MacGregor's cases were all under 3 weeks, the ages at death being 16, 9, 9, 7, 5, and 4 days in the uncomplicated cases. In two others death occurred at 10 days and 6 hours respectively, following an infection of a meningocele in spina bifida.

In the present series, all were cases of primary meningitis except possibly Case 4, where infection of the mastoid air-cells may have preceded the meningitis. All deaths occurred under 3 weeks of age, at 17 hours, 8, 14, 11, 16, and 4 days. Case 1 is of particular interest, as there does not appear to be any previous record in the literature of a death from primary suppurative meningitis occurring less than 24 hours after delivery. In this incidence infection most probably occurred *in utero* at the time labour was induced.

Both Craig (1936) and Cruickshank state that infants developing meningitis are often premature. Two of the present cases were born prematurely. They were from a family of quadruplets, and it is well recognized that such infants born in multiple pregnancies possess a lower resistance to infection than those born at full term and of a single pregnancy. Of the two remaining children in this family, one, who had a rise of temperature during the second week of life, died of hydrocephalus at the age of 6 months. This condition may well have been secondary to unrecognized meningitis in the neonatal period, although no necropsy was performed to confirm it. The fourth child is alive and well and now 3 years old.

Symptomatology and diagnosis.—During the newborn period and in early infancy meningitis presents few, if any, symptoms which are referable to the local condition. The onset may be sudden or gradual. The temperature frequently remains normal, as was the case in 25 per cent of Levinson's series. However, a sudden rise of temperature may be an early sign, and this was observed in four of the six cases here reported. Other early signs are failure to feed and restlessness (three cases) and twitching of limbs or muscular spasms (three cases). Neck rigidity is not common and was only observed in Case 2. Convulsions frequently occur before death, and a cephalic cry may develop. Bulging of the fontanelle was observed in only one case, although Levinson states that it is nearly always present. All these signs are suggestive of an intracranial lesion or at any rate cerebral anoxia, which may also be produced by atelectasis, neonatal pneumonia, septicaemia, and neonatal tetani. Under these circumstances it is important that immediate examination of the cerebrospinal fluid following lumbar puncture should be performed. This will confirm or deny any suspicion of the possibility of an intracranial lesion being present. It will also enable a differential diagnosis to be made as between meningitis and intracranial subdural haemorrhage.

In addition, Fothergill and Sweet (1933), having isolated the organism from the blood as well as the cerebrospinal fluid in 88 per cent of cases occurring in infants between 1 and 5 weeks old, state that bacteriaemia is very common in this type of meningitis. In this series the occurrence of bacilli in the blood was demonstrated in Case 4, where a macrophage containing ingested bacteria was seen in blood film (Plate I*b*).

Bacteriology.—It is probable that in all the six cases outlined above the responsible organism was *Bact. coli*, and a certain bacteriological diagnosis was made in five of them, as it is extremely unlikely that the presence of coliforms isolated was due to post-mortem infection of tissues. There have been two reviews where the bacteriology of neonatal meningitis has been classified—that of Cruickshank (1930) and a later one of Flensborg (1942), which includes all cases in the literature at that time. To these should be added MacGregor's cases (see Table).

TABLE

ANALYSIS OF CASES OF NEONATAL MENINGITIS IN THE LITERATURE

	Cruickshank (1930)	Flensborg (1942)	MacGregor (1946)
Bact. coli ..	11	68	6
Staph.	1	9	—
Strep. ..	7	23	—
Staph. + *Strep.*	—	7	—
Pneumococcus	3	12	1
H. influenzae ..	—	4	—
N. catarrhalis ..	—	1	—
Gonococcus ..	—	1	—
Mixed flora ..	—	4	—
Ps. pyocyaneus	1	—	—

In contrast Neal (1926) reviewed fifty cases of meningitis occurring in children over three weeks and under three months of age. The incidence of the various organisms in thirty-five cases where bacteriological diagnosis was made was as follows

N. meningococcus:	24 cases
M. Tuberculosis:	5 cases
Pneumococcus:	3 cases
Bact. coli:	3 cases

From the findings in all these reviews, coliforms are clearly the commonest pathogen in neonatal

meningitis. However, its incidence decreases with age and it is rarely found over two years, when meningococcal meningitis is reaching its maximal incidence. Although the most susceptible age period for this infection is given from 0 to 5 years (Compton, 1918), it is unusual in the first three months of life (Topley and Wilson, 1946). A possible reason for its low incidence may be found in the possession of a congenital immunity which lasts only for a few months. The restricted age incidence of coliform meningitis is not easily explained, but some light may be thrown on this point by a study of specific agglutinins present, in the sera of children at birth, to these and similar organisms.

Ravid (1935) says that "in the blood of foetuses and the newborn agglutinins to *B. coli* are absent," but he presents no evidence to substantiate this statement. Flensborg in his review also states that the newborn lack normal coli agglutinins present in adults.

Recently Wright (1947) has studied the transmission of coli agglutinins across the placenta, using seven different suspensions of coliforms obtained from "pathological" lesions. She found: (1) that the titres of both "O" and "H" agglutinins in the mother's serum at the time of delivery corresponded closely to that in the cord blood of the infant; (2) that of 55 mothers and babies examined, practically none possessed agglutinins to all the seven suspensions, and one or two no agglutinins at all. Most of the subjects possessed recognizable agglutinins to about half the suspensions used.

From her results it seems likely that nearly every infant is vulnerable to any strain of coliform for which little or no agglutinins are present, and that chance contacts with such strains may result in infection. This susceptibility, together with the well recognized feebleness of antibody production during the early months of life, may explain the high incidence of coliform meningitis at this age.

Pathology.—The pathological changes in these cases differ little from those seen in other types of suppurative meningitis except for the predominance of lymphocytes and mononuclear cells in the exudate. In most of the infants the meningitis probably followed bacteriaemia. The portal of entry of the responsible organisms is, however, uncertain clinically, and the post-mortem findings give no further assistance in locating the site of primary infection.

Treatment.—Barrett and others (1942) say that the mortality without chemotherapy is 80 per cent, and none of the cases in the present series recovered. They review six cases in which sulphonamides were used. There was only one death in the acute phase, although at least one other appeared to have died later from hydrocephalus. They say that sulphathiazole was then the drug of choice.

Since then, Pearlman and Bell (1944) have recorded a death in neonatal meningitis following adequate treatment with various sulphonamides in a newborn child, and Kohlbry (1942) a recovery in a male child aged 7 days treated with sulphapyridine. In an older patient Alexander (1946) treated successfully a coli meningitis following extensive fractures and osteitis of the right tibia (male, aged 19 years), with streptomycin.

As the organism in Case 6 was sensitive to 100 units per ml. of penicillin, yet another possible line of treatment was opened up by giving a large dose of the drug intrathecally.

However, the number of treated cases is still very small and it is hoped that, with earlier diagnosis in the future, these methods of chemotherapy may be more fully tested.

Summary

The literature on neonatal meningitis is reviewed and a series of six fatal cases described.

The aetiology, symptomatology, and bacteriology are discussed, views being presented to explain the predominance of coliforms as the responsible pathogen. Possible lines of treatment are discussed.

My thanks are due to Prof. G. Payling Wright and Dr. Philip Evans for helpful criticism in the preparation of this paper, to members of the clinical and pathological staff of Lewisham L.C.C. Hospital for help with notes of three of the cases, and to R. S. Morgan for the photomicrographs.

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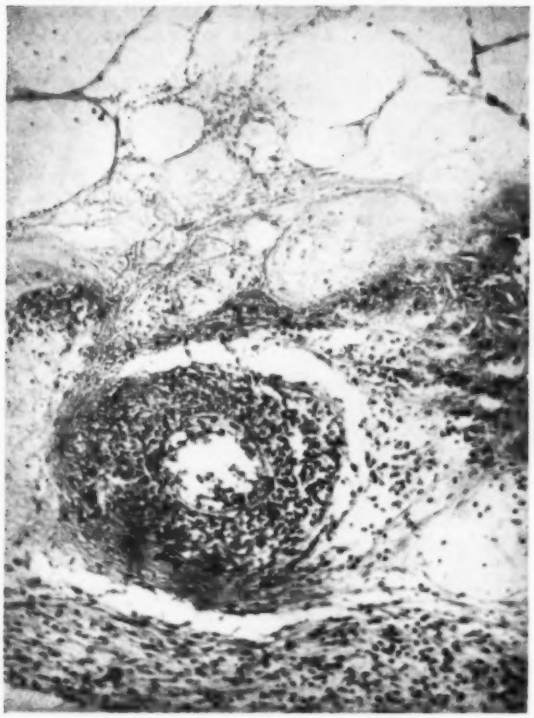
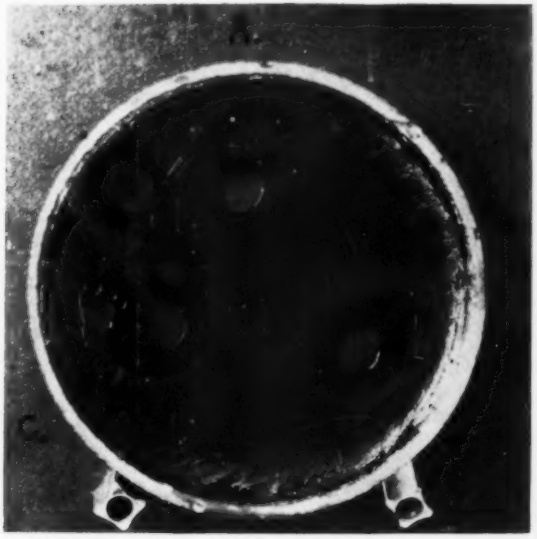


(*a*).—Section through the spinal meninges of a case of tuberculous meningitis, showing the fibrin masses in the upper half of the picture



(*b*).—Areas of clearance of test organism produced by streptomycin plus heparin (C) and streptomycin alone (B). No clearance round heparin alone (A).

PLATE II

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J. clin. Path. (1948), **1**, 157.

THE USE OF INTRATHECAL HEPARIN IN CONJUNCTION WITH STREPTOMYCIN IN THE TREATMENT OF TUBERCULOUS MENINGITIS

PRELIMINARY REPORT

BY

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One of the commonest causes of failure in the treatment of tuberculous meningitis by streptomycin is the formation of intracranial and intraspinal adhesions with resultant hydrocephalus. These adhesions are produced partly as a result of the fibroblastic reaction that occurs around tuberculous foci, but their inception and growth may well be stimulated by the deposition of fibrin from the cerebrospinal fluid which is rich in fibrinogen in this condition. The photomicrograph (Plate II*a*) illustrates that fibrin is, in fact, laid down in this condition. This was taken from the spinal cord of a child who had died from tuberculous meningitis after several weeks of treatment by streptomycin, and the masses of fibrin in the upper part of the picture can be clearly seen. If fibrin deposition is of importance in the formation of tuberculous adhesions, therefore, it might reasonably be hoped that the prognosis would improve if this could be prevented.

The obvious drug for this purpose is heparin. We decided for this reason to investigate the possibility of introducing this substance into the cerebrospinal fluid with streptomycin, and to attempt to assess its value in the treatment of tuberculous meningitis. Our preliminary findings on the interaction of streptomycin and heparin appeared to be of sufficient interest to warrant this preliminary communication. The investigations so far completed, and the conclusions derived therefrom, are given below.

Effects of Admixture of Heparin and Streptomycin Solutions

When a solution of heparin ("liquemin," or "heparin Boots," or "heparin Evans") was added to one of commercial streptomycin (hydrochloride or calcium complex), a precipitate was slowly formed. The nature of this precipitate has not yet been fully investigated, but certain properties have been noted. Examination of the supernatant fluid after centrifugalization revealed that there was no diminution of the streptomycin content during this reaction. Streptomycin assays were carried out both by serial dilutions in fluid media and cup plate methods.

The results of one of the latter methods of assay are shown in Plate II*b*. In this experiment, one drop of streptomycin solution of strength 25 units per ml. was added in the cup C to one drop of heparin solution of strength 1,000 units per ml., thus ensuring that a very large excess of the latter was present. The photograph shows that there was no diminution of the area of clearance of the test organism by this mixture as compared with the control cup B, into which was placed one drop of the same streptomycin solution and one drop of saline. Into cup A was placed one drop of a 1,000 unit per ml. heparin solution in order to demonstrate that it has no bacteriostatic powers.

Solutions of known strengths of heparin and streptomycin were again mixed and, after centrifugalization, the heparin contents of the supernatant fluid and of the deposit were estimated.

In the earlier stages of the work this heparin content was assayed on the basis of its delaying powers upon the deposition of fibrin from human plasma in the presence of excess thrombokinase and adequate calcium. This method is based on the assumption that heparin exerts its influence by the inactivation of thrombin and prothrombin (Best and Taylor, 1945). As McIntosh (1941) has pointed out, however, this method tends to give inconsistent results so that it was later replaced by a slight modification of the toluidine blue method of Trethewie and Melvin (1945). We have compared the colours of the unknown heparin toluidine blue mixture with that of the standard in a colorimeter instead of in comparator tubes as described by them. This method is quicker and gives consistent results but, of course, measures heparin in colour units.

These assays showed that:

(*a*).—If the amount of heparin in solution (in mg.) was less than approximately two-thirds of the amount of streptomycin (also in mg.) to which it was added, then no heparin was present in the supernatant fluid after removal of the precipitate. This was true whether the heparin was assayed by the toluidine blue method or by its anticoagulatory effect. This applies to the commercial preparations of heparin used by us, which contained between 90 and 110 units of heparin per mg.

(*b*).—If more heparin was then added to the mixture a further precipitation occurred, but active heparin began to appear in the supernatant fluid, so that by adding sufficient excess of heparin, concentrations of the order of 1,000 units per ml. or higher could be obtained free in the clear supernatant fluid in the presence of streptomycin. Even when these concentrations of heparin had been obtained, the addition of further heparin to the mixture still produced a precipitate. The addition of more streptomycin, on the other hand, did not cause a precipitate. Thus it was possible to prepare a mixture of heparin and streptomycin in clear solution and high concentration which could be introduced into the theca even though previously injected streptomycin was still present, without the danger that an intrathecal precipitation might occur.

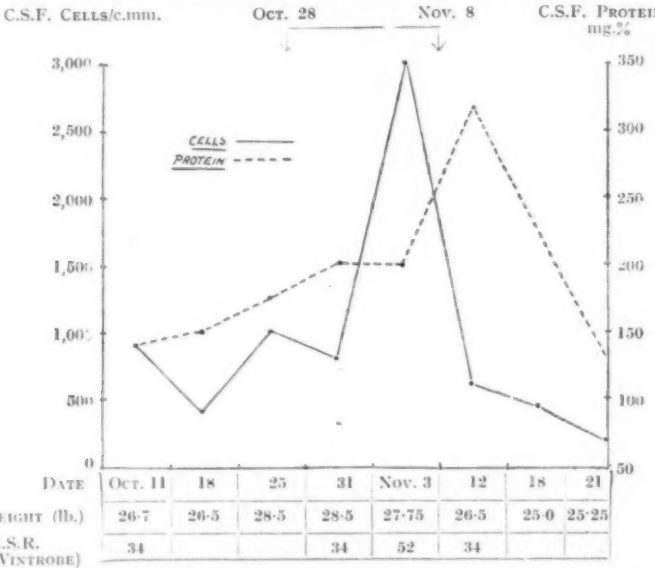
(*c*).—The precipitate, which consisted of a mixture of a white powdery material and a black tarry substance, contained active heparin as revealed both by the toluidine blue method and its anticoagulatory effect. As no streptomycin was lost from solution, there was presumably no streptomycin in the precipitate. Thus the precipitate must have been produced by a reaction between heparin and some impurity in the streptomycin.

(*d*).—All the heparin lost from the supernatant fluid was found to be present in the precipitate.

(*e*).—The precipitate showed slight solubility when warmed.

Effect of Introducing Heparin into the Theca of a Patient receiving Intrathecal Streptomycin

This was only tried on one patient, owing to the adverse reaction obtained. One ml. of a 1,000 unit per ml. solution of heparin was introduced into the theca daily, immediately after the introduction of streptomycin, and at the end of the twenty-four-hour period the streptomycin and heparin contents of the cerebrospinal fluid were assayed. The changes in the cerebrospinal fluid and the reaction of the patient are given in the composite graph



Date	Oct. 11	18	25	31	Nov. 3	12	18	21
Weight (lb.)	26·7	26·5	28·5	28·5	27·75	26·5	25·0	25·25
E.S.R. (1 Hr. Wintrobe)	34			34	52	34		

FIG. 1.—Changes in the cerebrospinal fluid, weight, and erythrocyte sedimentation rate during a course of daily intrathecal injections of heparin and streptomycin given separately. From Oct. 28 to Nov. 8 the patient had been receiving intrathecal and intramuscular streptomycin.

(Fig. 1). As can be seen from this, the white cells and protein contents of the cerebrospinal fluid rose sharply to 3,000 per c.mm. and 320 mg. per 100 ml. respectively. Coinciding with this, the patient began to show neck rigidity and incontinence with increased irritability and anorexia, whilst his weight, which had previously been increasing, started to decrease. On several occasions he showed evidence of experiencing pain immediately after the heparin injections, but it was impossible to confirm this as he was not co-operative.

This single case suggested that this method of giving heparin was not practicable. A combined solution of heparin and streptomycin was therefore prepared by adding 80,000 units of heparin in 10 ml. of saline to 1,000,000 units (1 g.) of streptomycin in an equal volume of saline. The mixture was allowed to stand in the refrigerator overnight to allow the maximum deposition of precipitate. The clear supernatant fluid was then decanted. The streptomycin content of this clear fluid was 50,000 units per ml., whilst the heparin content was found to be approximately 1,000 units per ml. This was injected in 2.0 ml. doses intrathecally into the same patient who had previously reacted adversely to the separate injections of streptomycin and heparin. The graph (Fig. 2) shows that the reaction produced was slight. We were thus encouraged to repeat the process in two other patients who had just been admitted and who had received no streptomycin previously. The changes in the cerebrospinal fluid of one of these are shown in Fig. 3 and, as can be seen, there was very little adverse reaction. The dose of free heparin was later increased to 5,000 units per dose in order to produce a residual concentration of between 1 and 2 units of heparin per ml. of cerebrospinal fluid at the end of the twenty-four-hour period.

Effect upon Heparin of the Streptomycin of the Cerebrospinal Fluid, following Purely Intramuscular Injections of this Latter Substance

It would appear probable that the precipitate formed by the admixture of solutions of heparin

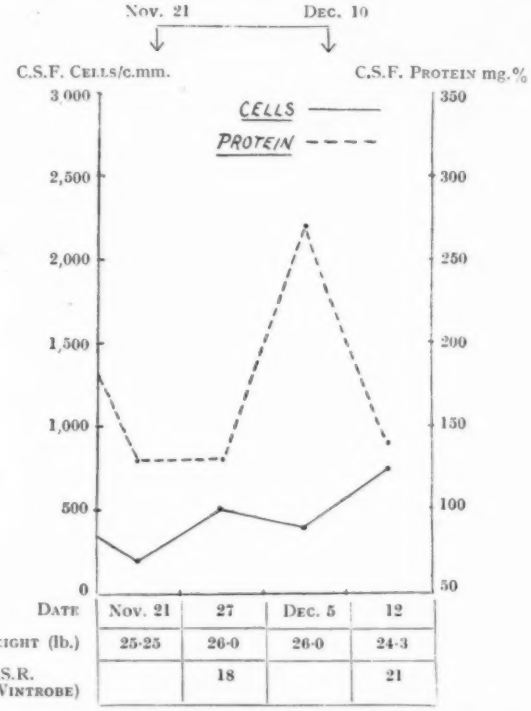


Fig. 2.—Cerebrospinal fluid changes during a course of daily injections of heparin–streptomycin mixture from Nov. 21 to Dec. 10. Case 1.

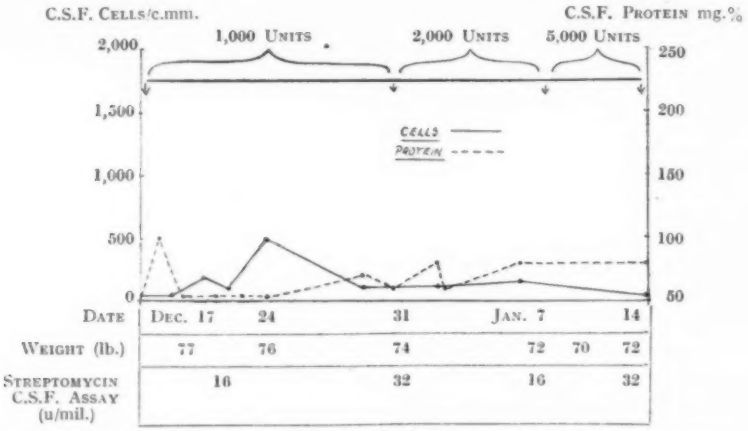


FIG. 3.—Cerebrospinal fluid changes during the daily administration of heparin–streptomycin mixture containing a higher concentration of heparin than in Case I.

and streptomycin is caused by impurities in the streptomycin. It was decided, therefore, to determine whether streptomycin that has been given intramuscularly and has reached the cerebrospinal fluid via the blood stream has a similar effect upon heparin solutions, that is, the production of a precipitate. Specimens of cerebrospinal fluid were obtained from patients receiving streptomycin by the intramuscular route only. These were withdrawn about one hour after the intramuscular streptomycin had been given, so that the concentration of the drug should be at a maximum. Equal quantities of this and of known strengths of heparin solutions were mixed, allowed to stand for twelve hours, centrifugalized, and the surviving heparin assayed. It was found that in all cases a small proportion of the heparin was lost, but that this was not related to the amount of streptomycin present in the cerebrospinal fluid samples. It may well be that the losses that occurred were due to fixation of heparin by fibrin precursors of the cerebrospinal fluid.

Effect of Injecting Heparin into the Theca of Patients receiving Intramuscular Streptomycin Only

The evidence derived from the preceding experiment suggested that no adverse reaction would

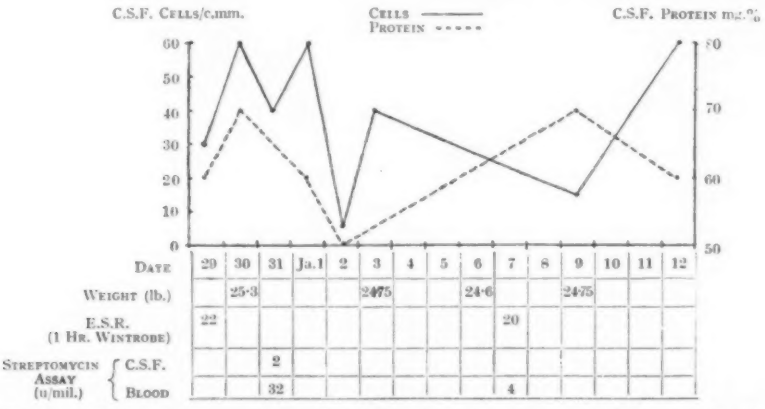


FIG. 4.—Changes in the cerebrospinal fluid during a course of daily intrathecal injections of heparin in a patient on intramuscular streptomycin.

follow the intrathecal administration of heparin to a patient who was receiving streptomycin by the intramuscular route only. Heparin was introduced, therefore, in 2,000-unit doses daily into the theca of a patient receiving this form of therapy, and changes in his cerebrospinal fluid and clinical condition were noted. The graph (Fig. 4) shows that no significant adverse reaction occurred.

Conclusions

1. The admixture of streptomycin and heparin causes the appearance of a precipitate which removes a large proportion of the heparin from solution, but has no effect on the streptomycin.

2. The occurrence of this reaction in the spinal theca produces an adverse effect on the patient.

3. A mixture of streptomycin and heparin in clear solution can be prepared which can be introduced with safety into the theca and gives comparable streptomycin assays with those found if streptomycin is given alone.

4. When the patient is being given intramuscular streptomycin only, heparin can be introduced into the theca without causing a reaction.

By this means it is possible to inject heparin regularly into the thecae of patients suffering from tuberculous meningitis under streptomycin treatment in an attempt to delay or inhibit the formation of adhesions. The effect of this on prognosis has yet to be investigated.

Addendum

Since sending this article to press we have been receiving samples of very pure heparin from the Evans Biological Institute, Runcorn. Very little precipitation results from the admixture of solutions of this heparin and streptomycin, and the supernatant fluid contains a very high concentration of heparin. This has resulted in a saving of heparin, but accentuates the need for assaying the heparin content of all heparin/streptomycin mixtures before use.

Our thanks are due to Dr. A. C. T. Vaughan for the streptomycin assays of cerebrospinal fluids; to Dr. R. R. Hughes for permission to investigate his patients; to Dr. J. M. Swithinbank for his willing co-operation in treating the cases; and to Drs. O. F. Thomas and A. B. Christie for providing beds for the patients under investigation. The streptomycin used in this work was provided by the Streptomycin Committee of the Ministry of Health.

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A STANDARDIZED MODIFICATION OF THE LAUGHLEN TEST FOR SYPHILIS

BY

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(RECEIVED FOR PUBLICATION, DECEMBER, 1947)

Laughlen published the first account of his test for syphilis in 1935.

The antigen was made thus: to 1 ml. of Kahn antigen was added a "small quantity" of a fat stain (Scharlach R or Sudan III) and four drops of tinct. benzoini co., B.P. The mixture was warmed in a water-bath at 50° C., and 10 ml. of 1.5 per cent saline, also warmed to 50° C., was added. This pink, milky-looking fluid was "sensitized" by the addition of varying amounts of 9 per cent salt solution. For the test one drop of this sensitized antigen was mixed on a slide with one drop of the serum under examination and gently rocked for 10 minutes; precipitation occurring within this time indicated a positive result. Several modifications of this technique have been described by various workers.

The Present Study

The present study was undertaken with a view to finding a simple method of making a reliable Laughlen antigen which was capable of being scientifically reproduced in a standard manner. The components of the antigen were first considered individually.

Kahn antigen.—Experiments with Kahn antigens from several sources showed that the one produced by Burroughs Wellcome and Co. was highly sensitive—it was used in all subsequent work and proved satisfactory.

Stains.—The "knifepointfuls" and "small quantities" referred to in some versions of the Laughlen test are too vague for accurate work. It was found that a simple method of overcoming the difficulty of the very small amount of stain required was to use a 3 per cent alcoholic solution made as follows: to 100 ml. of absolute ethyl alcohol in a 250 ml. flask is added 3 g. of the stain powder. The flask, stoppered tightly with cotton wool covered with tinfoil, is shaken for 5 minutes in a Kahn "shaker" and placed in an incubator at 37° C. for 30 minutes. The resulting solution is dark red but contains a thick deposit of insoluble material, which is removed by filtering twice through Whatman's No. 1 paper. The volume is made up to 100 ml. with absolute alcohol. By using this solution the stain may now be accurately measured, 0.1 ml. being added to 1 ml. of Kahn antigen. This amount of stain solution has no adverse effect on the final Laughlen antigen. Of several fat stains tried, Scharlach R was found to be the best.

The diluting saline.—Better results were obtained with the Laughlen test when the diluting saline was buffered, with optimum value at *p*H 6.6. The buffer-saline consists of 9 g. NaCl added to 1 litre of M/15 Sorensen's phosphates at *p*H 6.6. Pyrex glass is used throughout, as a precaution against increasing alkalinity. The figure mentioned above, 0.9 per cent, has been found to be the optimum NaCl concentration at *p*H 6.6. With buffer-saline it is to be noted that alteration of the salt content does not produce the same wide variations in antigen sensitivity as is found with ordinary saline.

The benzoin.—A 1-ml. pipette delivers 0.3 ml. (the optimum quantity) tinct. benzoini co. more accurately than "drops."

Preparation of Antigen

Into a Pyrex test-tube (6 in.×5/8 in.) pipette is put 10 ml. of buffer-saline. The tube is placed in a water-bath at 50° C. and left while the other components of the antigen are prepared. To 1 ml. of Kahn antigen (Burroughs Wellcome and Co.) in a similar tube is added 0.1 ml. of the 3 per cent stain solution and 0.3 ml. tinct. benzoini co., and this is placed in the water-bath for 2 minutes. Both tubes are removed and the contents mixed by pouring the saline into the antigen, back and forwards four times. The resulting pink liquid is the modified Laughlen antigen. Sedimentation, which occurs within a few hours, does not impair the qualities of the antigen: before use the colloidal appearance is restored by inverting the container. The sensitivity of the completed antigen is low when first made, but rises to its maximum within the next 30 to 36 hours, thereafter remaining constant for about four months. It should, therefore, be laid aside for about 36 hours before testing. Further sensitization by strong saline is unnecessary. The antigen is stored at room temperature.

The Apparatus

The mechanical mixer (Plate III*a*) consists of a circular porcelain plate, marked in 3/4-in. diameter

circles with black enamel, mounted for rotation at 20° to the horizontal on an adapted gramophone motor.

Two Dreyer pipettes are needed, one for the antigen and one for sera. They are each calibrated to deliver 0.05 ml. serum per drop.

THE TEST

The sera are inactivated as usual. The need for inactivation has been questioned by several workers using the Laughlen test, but experience with the present modification has shown it to be necessary (Table I).

Method.—Tube racks are designed to take 12 specimens per row; it is convenient to have two-row racks, each row of which is given a reference letter (A, B, etc.) which is marked on the plate as shown in Plate III*a*. Using the appropriate pipette, rinsed out once with saline between specimens, one drop (0.05 ml.) of each serum is placed in its respective circle, working from left to right, and starting with the outer six circles. The next row is pipetted on to the other half of the plate. Into each circle is now added one drop of antigen, which is mixed by inversion just before use. The motor of the "mixer" is wound up and the plate placed on the turntable, which is set going at the standard speed of 18 to 20 revolutions per minute for 10 minutes. This "standard speed" is empirical and is simply that which makes for smooth mixing of the contents of the circles without causing overflowing. (The speed control of the motor may need adjustment to obtain this slow rate.) The mixtures of antigen and serum should fill each circle and flow round smoothly inside the margins, but if any remains as a blob of liquid the motor should be stopped momentarily and with a piece of thin wire the fluid may be spread out gently to fill the circle's area. At the end of the 10 minutes the plate is removed from the turntable and the twenty-four tests examined by means of a ×6 hand lens. The following results may be found:

Negative.—Appearance uniformly colloidal. No precipitate visible even with the lens.

Doubtful (+).—A very faint precipitate visible with the lens. Of no *diagnostic* importance.

Positive (++).—A definite, fine precipitate, visible to the naked eye. Does not indicate syphilis *per se*, and requires confirmation by Wassermann and or Kahn reactions.

Positive (+++).—A heavy precipitate. Indicates syphilis in 95 per cent of cases.

Positive (++++).—A very heavy precipitate, concentrated into roughly twelve "clumps," always indicates syphilis and is invariably accompanied by a ++ W.R. and a ++, +++, or ++++ Kahn.

The various degrees of positive precipitation are shown diagrammatically in Plate III*b*.

TABLE I

RESULTS OBTAINED WITH 75 SERA BEFORE AND AFTER INACTIVATION

	Modified Laughlen							
			Before inactivation			After inactivation		
	W.R.	Kahn	++, +++ or ++++	+	—	++, +++ or ++++	+	—
22	—	—	—	—	22	—	—	22
16	—	++	4	7	5	15	—	1*
8	±	++	—	6	2	8	—	—
4	+	++	1	2	1	4	—	—
18	++	++	6	9	3	17	1*	—
5	++	+++	4	1	—	5	—	—
2	++	+++	2	—	—	2	—	—
75	(total)							

* Treated case.

TABLE II

ANALYSIS OF 521 DISCREPANCIES OBTAINED WITH UNTREATED CASES

Initial test				Three weeks later				No. of sera not obtained
No. of sera	W.R.	Kahn	M.L.	No. of sera	W.R.	Kahn	M.L.	
260	—	++	—	6	—	++	—	51
				203	—	—	—	
24	±	—	—	22	—	—	—	2
155	—	—	+	64	—	—	—	91
32	—	—	++	3	++	++	+++	2
				17	—	—	—	
				8	—	—	+	
				2	±	++	++	
34	±	+	++	2	++	+++	+++	23
				9	±	+	++	
16	—	+	++	1	++	++	+++	3
				12	—	—	—	
521	(total			349				172

M.L.—Modified Laughlen.

Results

The above modification of the Laughlen reaction has been tested with 16,824 sera, in parallel with the Wassermann (Wyler, 1929, 1931) and Kahn tests. Of these, 11,496 were diagnostic specimens and 5,328 from cases undergoing antisyphilitic treatment. In the former group, all three tests gave negative results with 374, while 521 sera produced discordant results of one kind or another. These discrepancies have been analysed (Table II), and are discussed later. In the treated group, 1,460 specimens were positive with the three tests, and 2,448 negative. 1,420 sera gave discrepancies which are analysed in Table III.

Discussion

This modified Laughlen (M.L.) test is intended mainly for use as a quick and convenient screen test. To be useful, its sensitivity level must be such that no serum from a new, untreated case escapes detection, even though it may be classed as doubtful or weakly positive by other tests for syphilis. If the M.L. test is to be used for the control of treatment, its sensitivity must in every instance be at least as high as that of any standard test. Non-specific positives are to be expected in both instances, but, if comparatively few in number, they are of little importance because all positive sera should be re-tested by the Wassermann test, the Kahn test, or both.

Accordingly, the results of the investigation may be discussed in relation to untreated and treated cases.

TABLE III

DISCREPANT RESULTS WITH SERA FROM 1,420 TREATED CASES

	W.R.	Kahn	M.L.
765	—	—	++
582	—	++	++
37	—	++	—
6	+	++	+
13	±	++	—
17	±	—	++
1,420 (total)			

M.L.—Modified Laughlen.

Untreated cases.—No serum giving a positive Wassermann reaction was negative by the M.L. test. Direct agreement between the two methods was found in 10,975 tests, plus 260 (Table II), a total of 11,235 or 97.7 per cent. If the 121 cases in which re-testing produced agreement are added to this total the percentage rises to 98.5, and it might be greater if more of the discrepant sera had been available for re-testing. In no instance was there any evidence of an earlier specific response to the Wassermann test than to the M.L. test, the 24 doubtful Wassermann results being accepted as non-specific on the evidence of the later tests. Of the 155 sera giving doubtful M.L. readings, all those available for re-testing were found negative, which confirms the belief that this degree of precipitation can usually be disregarded. Nevertheless it is a useful reaction to record, but does not imply that further examination of a serum is necessary unless this is indicated by suggestive clinical evidence.

A similar comparison with the Kahn test showed that no specific positive or "doubtful" result was ever found in a syphilitic serum giving a negative M.L. test. The large number, 260, of non-specific Kahn results in this series was due probably to the large number of sera from pregnant women. But if the doubtful M.L. results are disregarded substantial agreement was achieved with the Kahn test in 96 per cent of examinations, or in 98 per cent if the "three weeks later" results are taken into account.

Thus in a large series of serum examinations the M.L. test proved 100 per cent sensitive. Specificity in a screen test is of less moment. The number of non-specific positive M.L. results simply indicates the sera which have to be unnecessarily re-tested by Wassermann, Kahn, or both. In this series 82 positive M.L. reactions were associated with negative or doubtful Wassermann-Kahn results. Examination three weeks later showed that at least eight of such results were specific; therefore only 74 sera, approximately 0.7 per cent of the total, would have been re-tested unnecessarily by an accepted standard procedure had the M.L. test been used for screening. In one contingency, however, it may be desirable to know the actual non-specific rate of this test. If an immediate report is required—as, for instance, on a patient leaving the district, or on a blood donor prior to a direct transfusion—it may be helpful to know what reliance can be placed on this quick and convenient test. Its non-specific rate here was approximately 12 per cent. The Kline exclusion test has a non-specific rate of 9 per cent and the Hinton

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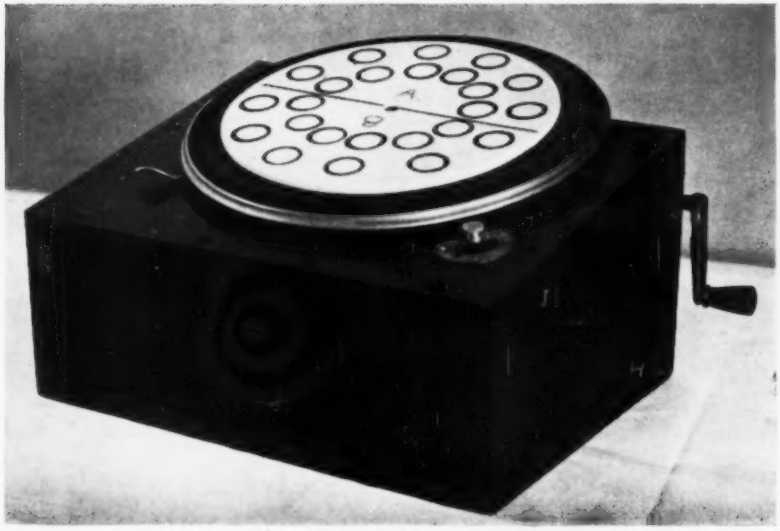
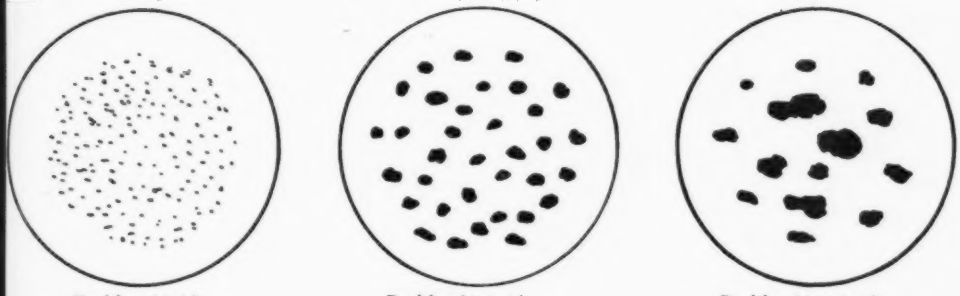


(*a*).—The mechanical mixer.



Positive (++). Positive (+++). Positive (++++).

(*b*) The various degrees of positive precipitation. All about ×2 magnification.

PLATE III

and Kahn presumptive test approximately 5 per cent. But in this series the non-specific rate of the standard Kahn was unusually high at 10 per cent, so that the high rate of the M.L. was probably due more to the nature of the material examined than to any deficiency in the test. Nevertheless this finding supports the belief that complete sensitivity and specificity are not attainable in any one simple test. Should a report be required at short notice it is, however, permissible to use the M.L. test if only complete precipitation, the + + + + reading, be accepted as positive, and all intermediate grades of precipitation classed as doubtful. All positive and negative readings will then be entirely specific.

Treated cases.—Complete agreement with the Wassermann was recorded in approximately 73 per cent of the specimens examined, and this low rate of agreement between the two tests was due to the many instances where the M.L. remained positive after the Wassermann and Kahn had become negative (Table III). On the other hand, 13 sera which were positive in varying degrees with both Wassermann and Kahn, and 37 sera positive with Kahn only, gave negative results with the M.L. Although the Laughlen reaction usually remains positive after the Wassermann and Kahn have become negative, about 1 per cent of treated cases react negatively to the Laughlen while the other tests are still showing a positive result. This irregular sensitivity, and especially the poor correlation with the Kahn test, so widely applied to the control of antisyphilitic treatment, suggests that this version of the Laughlen test is unsuitable for use with treated cases.

Summary

The composition of the Laughlen antigen and the technique of performing the test have been investigated. From the findings a modified procedure has been evolved, which has been tested in the examination of 16,824 sera, 5,328 from treated cases, 11,496 from untreated cases. The findings suggest that the modified Laughlen test has no special merits as a final diagnostic test, that it is not suitable for the control of treated cases, but that it is especially valuable as a " screen " test for diagnostic specimens. The degree of sensitivity is such that no syphilitic serum is missed, yet comparatively few non-specific positives are encountered. The antigen is easy to prepare by a standardized method and gives constant results for at least four months from its date of manufacture. The whole test is extremely simple to perform.

I desire to express my thanks to Prof. W. J. Tulloch, University College, Dundee, for his explanation of many points connected with the Laughlen reaction, and to Dr. R. D. Stuart, lately city bacteriologist, Glasgow, for his help and encouragement during this investigation. I should like also to acknowledge the assistance of many members of the staff of the Central Public Health Laboratory, Glasgow, without whose co-operation the investigation would not have been possible.

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SINGLE-SAMPLE TESTS IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE

BY

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Many attempts have been made to compare the usefulness of the various tests of liver function. The papers of White and others (1940), Wilson (1940), Higgins and others (1944), Maizels (1946), and Sherlock (1946) discuss the merits of such diverse estimations as bromsulphalein, hippuric acid, and urobilinogen excretion; bilirubin, cholesterol, cholesterol esters, differential proteins, fibrinogen, phosphatase, phospholipins, and prothrombin in serum or plasma; galactose and fructose tolerance; and the cephalin-cholesterol, colloidal red, Takata-Ara, and thymol turbidity tests. It has been difficult to find an independent measure of the degree of liver damage, and even the liver biopsy used by Sherlock and others may not always be satisfactory. If the tests are regarded as empirical aids in specific diagnostic problems, rather than as a means of measuring liver damage, the situation is simplified, though the evaluation of results must often depend upon the accuracy of clinical diagnosis.

The object of the present work has been to investigate the suggestion of Maclagan (1944a) that a combination of the thymol turbidity or the colloidal gold test with an estimation of the serum alkaline phosphatase would distinguish between infective hepatitis and obstructive jaundice in a high proportion of cases. Maclagan (1947) has published a survey of his results using these tests on two hundred cases of jaundice, of which fifty-six were obstructive and ninety-five infective hepatitis. He was able to distinguish between obstructive and non-obstructive jaundice in from 65 to 79 per cent of cases by combining one flocculation test with an estimation of the serum alkaline phosphatase. The limits set for the data to be considered of diagnostic significance were so severe as to exclude the possibility of misclassification within his group of cases. In practical work it is usual to accept some possibility of error as inevitable, and with this in mind Maclagan's results are very satisfactory. Rennie and Rae (1947) have reported their experience with the cephalin-cholesterol and colloidal gold tests in obstructive jaundice, infective hepatitis, and several other diseases involving liver injury. They showed that these two flocculation tests gave negative results in 84 per cent of cases of obstructive jaundice while positive flocculations were obtained with nearly as high a proportion of sera from patients with infective hepatitis. These authors point out that in spite of general agreement between the results of the two tests they were not always parallel, and a similar lack of strict correlation has been observed among the flocculation tests used in the present investigation.

In addition to the thymol turbidity and colloidal gold tests the present investigation has included Britton's (1945) modification of the Takata-Ara reaction, together with the estimation of the alkaline phosphatase, bilirubin, and albumin-globulin ratio. These six tests can all be performed on 3.5 ml. serum and have in this respect an advantage over the tolerance tests, which involve several venepunctures. The results have been analysed statistically by methods described by Bradford Hill (1946).

Experimental

METHODS

All colours and turbidities were read in a King (1942) photo-electric colorimeter, using Ilford spectrum light filters.

Takata-Ara reaction.—The modified method of Britton (1945) was used. Three tubes containing respectively 1 ml. of 1:2, 1:4, and 1:8 dilutions of serum in 0.85 per cent NaCl were treated with 0.25 ml. 10 per cent Na_2CO_3 and 0.3 ml. 0.5 per cent $HgCl_2$. The tubes were shaken after each addition and examined after twenty-four hours. The amount of precipitate in each tube was graded as 3, 2, 1, or 0, corresponding to the more usual notation of +, ±, trace, and 0. This device was adopted for statistical reasons, the result of the test being obtained by totalling the grades of the three tubes.

Colloidal gold test.—The method and grading of results were those of Maclagan (1944b).

Thymol turbidity test.—Maclagan's method (1944a) was used. The buffer made up as described by Maclagan gave a *p*H of less than 7.8, so the *p*H was adjusted to this value with NaOH. It was later found that this led to a reduction in sensitivity, but for purposes of comparison it was considered inadvisable to alter the procedure in the middle of the investigation.

Alkaline phosphatase.—This was estimated by the method of King and others (1942).

Differential serum proteins.—The biuret method of Robinson and Hogden (1940) was used for total proteins and albumin.

Bilirubin.—This was estimated by the method of King and others (1937).

Results

Normal Blood Donors

The three flocculation tests have been carried out on the sera of one hundred apparently normal blood donors, and the results are shown in Table I. (The large number of significant figures given in this and other tables are included for statistical purposes and not because they are supposed to have physical significance.) It has been assumed that the "normal range" is given by the mean ± twice the standard deviation. When a result outside the normal range was obtained with one of the flocculation tests on the serum of a normal blood donor the other two tests were usually normal.

TABLE I

RESULTS OF FLOCCULATION TESTS ON ONE HUNDRED SERA FROM NORMAL BLOOD DONORS

Test	Mean	Standard deviation	Mean ± 2 S.D.
Takata-Ara	0.4	0.92	0–2
Colloidal gold ..	0.3	0.81	0–2
Thymol turbidity ..	1.2	0.53	0.1–2.3

S.D. = standard deviation.

Flocculation tests and albumin-globulin ratio.—It has been stated by Bálint and Bálint (1942) that positive results in the Takata-Ara reaction are merely due to an abnormally low albumin-globulin ratio, and in particular to a relative excess of "euglobulin," which includes the fraction now referred to as γ-globulin. It was thought useful to compare the results of all three flocculation tests with those of differential serum protein estimations, and correlation coefficients have been calculated between the individual flocculation tests and between these tests and the albumin-globulin ratio. Pathological sera were used, and the results are shown in Table II.

TABLE II

CORRELATION COEFFICIENTS OF FLOCCULATION TESTS WITH ALBUMIN-GLOBULIN RATIO, AND OF FLOCCULATION TESTS WITH ONE ANOTHER

Correlation	Number of tests (n)	Correlation coefficient (r)	"Significance" $\left(r\Big/\sqrt{\frac{1}{n-1}}\right)$
Gold–A/G	187	0.22	3.0
Thymol–A/G	187	0.23	3.1
Takata–A/G	186	0.38	5.2
Takata–Thymol	352	+0.49	9.2
Takata–Gold	320	+0.61	10.8
Thymol–Gold	315	+0.77	13.7

The correlation coefficient divided by its standard error (col. 4) is in each case greater than 2 and is therefore significant. This does not prove, however, that positive flocculation tests are due to a common cause, and still less does it prove that the cause is a low albumin-globulin ratio. The lowest correlation coefficients in the table are those between the albumin-globulin ratio and the flocculation tests, and the highest correlation is between the colloidal gold and thymol turbidity tests. A reduced albumin-globulin ratio is more closely associated with a positive Takata-Ara reaction than with high results in the colloidal gold and thymol turbidity tests, and this point will be referred to in the discussion.

Infective Hepatitis and Obstructive Jaundice

Flocculation tests and proteins.—A comparison between results from patients with infective hepatitis, diagnosed clinically, with those from cases of obstructive jaundice proved at operation or necropsy, is shown in Table III. If a series of investigations had been done on the same patient the first complete set was used for the statistical analysis. It has been assumed that the difference between two means $(\bar{x}-\bar{y})$ is significant if $\bar{x}-\bar{y}$ divided by the standard error of the difference (S.E.) is greater than 2. While all 3 flocculation tests gave significant differences in the two forms of jaundice the colloidal gold and thymol turbidity tests gave much better discrimination than the Takata-Ara reaction.

The serum proteins and albumin-globulin ratio were almost identical in the two forms of jaundice.

Bilirubin and phosphatase.—The mean serum bilirubin in infective hepatitis (6.8 mg./100 ml.) was significantly lower than that observed in obstructive jaundice (12.2 mg./100 ml.), but the

TABLE III

COMPARISON BETWEEN RESULTS IN INFECTIVE HEPATITIS AND IN OBSTRUCTIVE JAUNDICE

		Takata-Ara (grade)	Colloidal gold (grade)	Thymol turbidity (units)	Total protein (%)	Albumin (%)	Globulin (%)	A/G ratio	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
Infective hepatitis	n	**37**	**32**	**37**	**28**	**28**	**28**	**28**	**38**	**36**
	$\bar{x}$	4.49	2.84	4.81	6.44	3.25	3.20	1.14	17.59	6.80
	S.D.	2.22	1.73	2.99	0.93	0.90	0.97	0.47	7.94	5.67
Obstructive jaundice	n	**34**	**31**	**34**	**25**	**25**	**25**	**25**	**33**	**34**
	$\bar{y}$	2.06	0.06	0.78	6.18	3.12	3.05	1.05	37.85	12.19
	S.D.	2.14	0.25	0.49	0.67	0.56	0.55	0.29	16.36	9.45
Analysis of differences	$\bar{x}-\bar{y}$	2.43	2.78	4.03	0.26	0.13	0.16	0.09	20.26	5.39
	S.E.	0.52	0.31	0.50	0.23	0.20	0.21	0.11	3.13	1.88
	$\frac{\bar{x}-\bar{y}}{S.E.}$	4.7	9.0	8.1	1.2	0.7	0.8	0.8	6.5	2.9

n = Number of patients. $\bar{x}$ and $\bar{y}$ = mean values. S.D. = standard deviation. S.E. = standard error of difference between means. A/G = albumin-globulin.

sc um alkaline phosphatase was of much greater discriminant value than the bilirubin.

Sherlock (1946) found that no positive correlation existed between the bilirubin and phosphatase contents of the serum in obstructive jaundice and infective hepatitis. This observation has been confirmed by the present work. In obstructive jaundice the correlation coefficient was 0.29, and the coefficient divided by its standard error was only 1.03, indicating that the correlation was not significant. The corresponding figures for infective hepatitis were 0.17 and 1.00. The number of cases on which these figures are based (72), combined with those of Sherlock, makes it virtually certain that bilirubin and phosphatase are not secreted into the serum by the same mechanism, which supports Maclagan's (1947) view that the rise in phosphatase "represents a positive secretory response of the liver cell to a variety of chemical or bacterial insults." An alternative explanation would be a difference in the mechanism of the removal of these substances from the plasma.

Relative discriminant value of tests.—If we regard $(\bar{x}-\bar{y})/S.E.$ as a measure of the relative discriminant values of the tests, the descending order of usefulness given by the figures in Table III is: colloidal gold, thymol turbidity, phosphatase, Takata-Ara, and bilirubin. Maclagan (1947) found that the alkaline phosphatase had the highest discriminant value, followed by thymol flocculation, colloidal gold, and thymol turbidity. Unfortunately the thymol flocculation was not recorded frequently enough in the present work for adequate statistical treatment.

N**

Miscellaneous Diseases

Although many pathological sera have been examined the number for each disease has been small except for infective hepatitis and obstructive jaundice. Malaria and infective mononucleosis, and to a lesser extent amoebic hepatitis and thyrotoxicosis, have given positive flocculation tests, while extensive carcinomatous metastases in the liver and almost total destruction of normal hepatic tissue in Banti's disease have given results within normal limits.

Discussion

Takata-Ara reaction.—In the series of eighty-one cases considered by Bálint and Bálint (1942), positive results in the Takata-Ara reaction were nearly always associated with a lowered albumin-globulin ratio. Only six anomalous results were obtained—four sera with albumin-globulin ratios of 1.3 to 1.6 gave positive results, whereas no flocculation was observed with two sera having albumin-globulin ratios of 1.1. Although the present results show a highly significant correlation (−0.38) between the Takata-Ara reaction and the albumin-globulin ratio, there were many examples in which the results were not parallel. Among 188 sera, twenty-eight had a Takata-Ara reaction lower than grade 2, coupled with albumin-globulin ratios below 1.2, and on the other hand Takata-Ara reactions higher than Grade 2 occurred with twenty-four sera having albumin-globulin ratios higher than 1.2. Britton's Takata-Ara reaction cannot therefore be regarded merely as a qualitative indication of lowered albumin-globulin ratio.

Colloidal gold and thymol turbidity tests.—There is disagreement concerning the mechanism of the flocculation tests, particularly regarding the thymol turbidity test. The evidence was reviewed by Maclagan and Bunn (1947), who found that γ-globulins were the main precipitating proteins in the thymol, gold, cephalin-cholesterol, and Takata-Ara tests. The cephalin-cholesterol and Takata-Ara reagents were also precipitated by α- and β-globulins from hepatitis sera. The gold test was inhibited by albumin and α- and β-globulins, while albumin inhibited the cephalin-cholesterol and Takata-Ara reactions. Cohen and Thompson (1947) believe that although the γ-globulins are raised in hepatitis sera while the β-globulins may only be slightly above normal, nevertheless a high thymol turbidity is probably due to a qualitative change in the β-globulin fraction. Unfortunately the conditions used by Cohen and Thompson were not the same as those specified by Maclagan.

The present investigation shows that the colloidal gold and thymol turbidity tests are not measures of exactly the same phenomenon. The correlation coefficient of + 0.77 between these two tests (Table II) is high, but in many individual cases they gave discordant results. Among 323 pathological sera, twenty gave abnormally high thymol turbidities combined with normal "golds," whereas eight had high "golds" with normal "thymols." Thus in about 9 per cent of these sera the results of the two tests were not in agreement, which would be unlikely if both depended on the same set of conditions. The flocculation is probably due in both cases to the same positive cause (e.g., γ-globulins), but the modifying factors such as albumin and α- and β-globulins are different (Maclagan and Bunn, 1947; Moore and others, 1945). The danger of drawing sweeping conclusions from comparisons between parallel results of these empirical tests is illustrated by the fact that slight variations in reagents and technique may give very different results with the same serum.

The technical difficulty of fractionating sera electrophoretically is a serious obstacle to quantitative investigation of the flocculation tests. The work of Maclagan and Bunn (1947) was based upon results from a single mixed specimen of "typical" hepatitis serum, and the limitation which this places upon their interpretations will be realized when it is considered that in a series of cases of "typical" infective hepatitis the results of the various flocculation tests may range from all being strongly positive to an occasional example in which all are negative (Tables IV and V).

TABLE IV

ANOMALOUS RESULTS IN INFECTIVE HEPATITIS

No.	Takata (grade)	Gold (grade)	Thymol (units)	A/G	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
1	1	1	1.8	2.1	10.0	5.5
2	2	0	0.9	0.9	15.8	4.3
3	0	0	0.4	2.4	33.2	6.1
4	3	0	1.4	1.2	13.6	1.4
5	0	1	1.8	—	17.4	6.0
6	0	—	1.1	—	19.6	3.6
7	3	0	3.2	1.4	16.5	21.0
8	5	0	—	1.5	13.4	—
9	5	3	2.1	1.3	16.4	9.7
10	2	2	2.1	1.6	9.7	4.1
11	3	—	1.6	—	12.5	4.6
12	5	—	2.1	—	24.0	2.5

Differential diagnosis of jaundice.—A comparison is shown in the Figure between the results obtained with the three flocculation tests when applied to sera from normal blood donors and from patients with obstructive jaundice and infective hepatitis. The means are shown at the corners of the triangles, and in the middle of each side is given the "significance" $(\bar{x} - \bar{y}/\text{S.E.})$ of the difference between the means at the corresponding corners. The Takata-Ara reaction in *both* forms

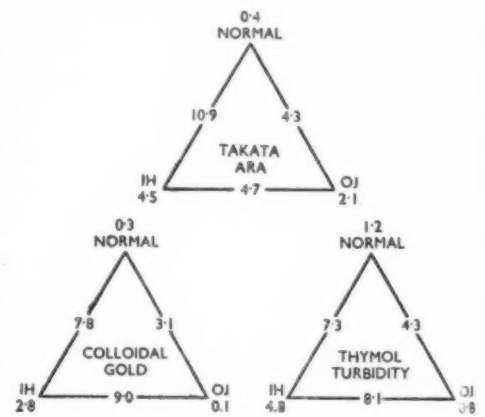

FIGURE.—Differences between results of flocculation tests in normal individuals and in patients with obstructive jaundice and infective hepatitis. (The figures at the corners of the triangles are means, and those on the sides represent significance of differences between means.)

of jaundice was significantly above the normal, which is consistent with the fact that liver damage occurs in both conditions. However, in the case of the thymol turbidity and colloidal gold tests the results in obstructive jaundice were significantly *lower* than the normal values. These two tests should not, therefore, be referred to as "liver function tests."

For the differentiation of obstructive jaundice from infective hepatitis these results suggest that in obstructive jaundice the colloidal gold test will be grade 0, or occasionally grade 1, the thymol turbidity 2.1 units or less, and the alkaline phosphatase greater than 25 units/100 ml. In infective hepatitis the colloidal gold will be greater than Grade 1, the thymol turbidity above 2.1 units, and the phosphatase less than 20 units/100 ml. No case of obstructive jaundice has been encountered which would have been wrongly diagnosed using either the colloidal gold or thymol turbidity test alone with the above criteria, but among thirty-nine cases diagnosed clinically as infective hepatitis no less than twelve gave misleading results with one of these flocculation tests (Table IV). In the first five cases shown in the table both tests gave results within normal limits. However, in ten of these twelve cases the phosphatase was below 20 units/100 ml., whereas among thirty-three cases of proved obstructive jaundice a phosphatase of less than 20 units/100 ml. only occurred five times, the lowest value observed being 16.8 units/100 ml. Taking all the criteria into consideration the only cases shown in Table IV which would be likely to be wrongly diagnosed are numbers 3, 6, and 12, and only in Case 3 are the results really typical of obstructive jaundice. Serial tests were done on seven of the twelve cases in Table IV, and three of these eventually gave positive flocculation tests.

The frequency of positive results for the Takata-Ara reaction in obstructive jaundice makes this test useless for the differentiation of the cases quoted in Table IV. Among thirty-three obstructive jaundice sera, fourteen gave results above Grade 2, and nine above Grade 3. It is noteworthy that the albumin-globulin ratios shown in the table are, with only one exception, within normal limits, which suggests that the liver damage could not have been severe, and in all cases except two the bilirubin is below the mean for infective hepatitis of 6.8 mg./100 ml.

That the chance of mis-classification, using the above criteria, is real and not due to mistaken clinical diagnosis is illustrated in Table V, which shows results from two soldiers, A and B, who entered hospital within a few days of one another. They were close friends and slept in adjoining beds in barracks, and there seems little doubt that they had infective hepatitis of common origin. The results of the tests on the two men were very different even when done simultaneously, and, if the colloidal gold and thymol turbidity results alone had been considered, A might well have been diagnosed as suffering from obstructive jaundice. The low phosphatase made such a diagnosis unlikely, but it must be admitted that it is a mistake to place too much reliance on this test when it conflicts with the flocculation tests. In doing so one would be discarding the results of two tests of high discriminant value in favour of that of a single test of lower discriminant value (Table III).

Maclagan (1947) gave a series of criteria for discrimination which excluded all possibility of mistaken diagnosis within his group of cases. A similar classification of the results obtained in the present work shows that, in round figures, all cases with phosphatase greater than 40 units/100 ml.

TABLE V

TWO CONTRASTED CASES OF INFECTIVE HEPATITIS, PROBABLY OF COMMON ORIGIN

Case	Date	Takata-Ara (grade)	Colloidal gold (grade)	Thymol turbidity (units)	Total protein (%)	Albumin (%)	Globulin (%)	A/G ratio	Alkaline phosphatase (units/100 ml.)	Bilirubin (mg./100 ml.)
A	1.8.46	1	1	1.8	8.4	5.7	2.7	2.1	10.0	11.1
	2.8.46	2	1	1.8	8.7	5.8	2.9	2.0	13.3	13.5
	7.8.46	1	0	1.2	—	—	—	—	—	—
	13.8.46	1	0	1.6	7.5	4.8	2.7	1.8	8.9	2.5
B	6.8.46	6	3	4.7	6.8	4.0	2.8	1.5	20.6	6.6
	7.8.46	6	4	4.8	—	—	—	—	—	15.0
	13.8.46	4	5	5.0	7.5	3.9	3.6	1.1	17.6	39.2
	24.8.46	4	1	2.4	6.7	3.8	2.9	1.7	13.6	4.1
	5.9.46	4	1	2.0	5.9	3.8	2.1	1.8	10.9	2.9

were obstructive jaundice and all with phosphatase less than 17 units/100 ml. were infective hepatitis. All sera having colloidal gold tests less than Grade 2 and thymol turbidities less than 2.2 units, combined with phosphatase higher than 33 units/100 ml., were from patients with obstructive jaundice, and all cases having colloidal gold tests greater than Grade 1 and thymol turbidities greater than 2.1 units were of infective hepatitis. These results agree well with those of Maclagan, except that Maclagan's thymol and gold reagents were evidently considerably more sensitive than those used in this investigation.

Summary

1. The usefulness of the Takata-Ara reaction, colloidal gold, and thymol turbidity tests, differential serum proteins, serum alkaline phosphatase, and serum bilirubin estimations for the differential diagnosis of infective hepatitis and obstructive jaundice has been investigated.

2. Statistical analysis indicates that all these tests except the differential serum proteins give significantly different results in the two forms of jaundice, but only the colloidal gold, thymol turbidity, and alkaline phosphatase are of high discriminant value.

3. The three flocculation tests are closely correlated with one another and, to a lesser extent, with the albumin-globulin ratio. Each is a measure of a different complex of factors, and none is merely a qualitative test for a lowered albumin-globulin ratio

4. The Takata-Ara reaction gave results above normal in both forms of jaundice, but the mean results of the colloidal gold and thymol turbidity tests were significantly lower in obstructive jaundice than in normal sera.

5. There is no significant correlation between the bilirubin and the phosphatase in either form of jaundice.

6. All sera from patients with obstructive jaundice had colloidal gold tests less than Grade 2 and thymol turbidities less than 2.1 units, and 75 per cent had a serum alkaline phosphatase greater than 25 units/100 ml. About 70 per cent. of sera from patients with infective hepatitis had colloidal gold tests greater than Grade 1, thymol turbidities greater than 2.1 units, and phosphatase less than 20 units/100 ml. All cases with phosphatase greater than 40 units/100 ml. were obstructive jaundice, and all with phosphatase less than 17 units/100 ml. were infective hepatitis. All cases with gold tests less than Grade 2 and thymol turbidities less than 2.2 units, combined with phosphatase higher than 31 units/100 ml., were obstructive jaundice.

Grateful acknowledgment is made to my medical colleagues at this hospital and at the Battle hospital, and particularly to Dr. W. Hausmann, for the supply of sera and reports. Drs. R. B. Fisher and P. White gave valuable advice on the interpretation of the statistics, and Mr. E. B. Love, B.Sc., carried out many of the estimations of phosphatase and bilirubin.

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TECHNICAL METHODS

THE ESTIMATION OF POTASSIUM BY A MICRO-METHOD

BY

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A method for the estimation of potassium using a silver cobaltinitrite complex was described previously (Cumings, 1939). The method as described below has overcome the disadvantages of the original method, which were the need for ashing sera and the rather difficult titration involved. The composition of the compound has been ascertained and will be indicated in this paper.

Breh and Gaebler (1930), after deproteinization of serum, added silver nitrate followed by the usual sodium cobaltinitrite reagent, and part of their procedure has been utilized. A colorimetric method is used in place of final titration for estimation of nitrite (Hoagland and Ward, 1942 ; see also Looney and Dyer, 1942).

Method

To 3.1 ml. of distilled water in a centrifuge tube are added 0.2 ml. serum followed by 0.3 ml. 2/3 *N*-H_2SO_4 and 0.3 ml. 10 per cent w/v $Na_2WO_4 2H_2O$; after mixing, 0.1 ml. of 5 per cent w/v $AgNO_3$ is added, and the contents mixed and centrifuged ; 2 ml. of the supernatant fluid are placed in a centrifuge tube graduated at 10 ml., and 1 ml. of my original silver cobaltinitrite reagent is added (Cumings, 1939), and the tube centrifuged after standing for one hour. To the precipitate are added about 10 ml. of 50 per cent acetone, which is centrifuged after mixing, and this procedure is repeated once more, after which 1 ml. of 2.5 *N*-NaOH is added to the precipitate and the tube placed in a boiling-water bath for five minutes. Distilled water is now added up to the 10 ml. mark. After mixing, the contents are centrifuged and 1 ml. of the supernatant fluid is diluted to 25 ml. with distilled water.

Five ml. and 10 ml. of this dilution are placed in test tubes made up to 10 ml. with water, and 1 ml. of 0.2 per cent sulphanilamide is added, followed by 0.2 ml. of 0.1 per cent. *N*-(1-Naphthyl)-ethylenediamine hydrochloride.

A blank of 10 ml. of distilled water, and two standards of 10 ml. of distilled water containing 0.004 and 0.008 mg. of sodium nitrite, are treated in the same way, and all tubes are placed in ice or in a refrigerator as at this temperature there is a greater constancy of colour production. After cooling 0.2 ml. of 6*N*-HCl are added to each tube, the contents mixed, and the tubes returned to the cold for fifteen minutes, after which time readings are taken in a colorimeter. (A King pattern photoelectric colorimeter has been found to be very suitable.) The pink to red colour is permanent for twenty-four hours.

A standard potassium solution should also be treated in the same manner as the serum by taking 0.2 ml. of a solution containing 51.7 mg. KNO_3 per 100 ml. (equivalent to 20 mg.K./100 ml.). With a visual colorimeter the calculation $\frac{S}{U} \times 20$ will give the result in mg./100 ml.

Results

Table I gives a few of the results obtained using this method, my original method, and that of King and others (1942).

TABLE I

COMPARISON OF RESULTS OF K IN SERUM BY THE METHOD OF KING AND OTHERS AND OUR METHODS

King	$K_3Ag_2Co_2Na(NO_2)_{12}$ method	
Mg./100 ml.	Original method mg./100 ml.	New method mg./100 ml.
18.0	21.0	19.5
25.3	26.6	26.6
24.1	24.0	25.0
18.0	19.0	20.7
27.0	25.6	26.3
20.4		21.6
20.6	21.3	21.6
19.2	21.6	19.8
19.0	17.0	18.0
40.0 (Haemolysed serum)	41.0	42.0

Composition of Compound

Samples of the potassium silver cobaltinitrite compound were prepared from known amounts of potassium nitrate, dried at 100° C., and weighed. Separate quantities of the compound so prepared were then analysed for potassium, silver, cobalt, sodium, and nitrite, and Tables II and III show the results obtained. The analytical methods used are indicated in the appendix.

TABLE II

MOLECULAR WEIGHT OF COMPOUND FORMED
(Assuming molecule contains 3 atoms K)

KNO_3 taken	Compound formed	Molecular weight
0.2636 g.	0.8695 g.	1020
0.0188 g.	0.0638 g.	1028

TABLE III

COMPOSITION OF COMPOUND

Constituent	Estimated (%)	Calculated (%)
K	10.94	11.40
Ag	20.1	21.05
Co	12.0	11.50
Na	2.2	2.24
NO_2	52.8	53.80

The results found by experiment and by calculation are in sufficiently close agreement to substantiate the view that the formula $K_3Ag_2Co_2Na(NO_2)_{12}$ with a molecular weight of 1026 is correct.

Discussion

The method presented is quick and reliable, and can be performed on 0.2 ml. quantities of serum.

If much less serum were used the amount of precipitate would be so small that any losses during centrifugation would cause a relatively large error.

The final pink colour is very easy to match and the method involved is an extremely delicate one for the estimation of nitrite.

Summary

A method for the estimation of potassium in small amounts of serum is described.

The potassium silver cobaltinitrite compound formed has been shown to have the formula $K_3Ag_2Co_2Na(NO_2)_{12}$.

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Appendix

ANALYTICAL METHODS USED

Sodium : King, 1946. *Micro-Analysis in Medical Biochemistry*, Churchill, London.

Cobalt :
1. Gravimetrically as $CoSO_4$.
2. Colorimetrically with choline chloride and sodium ferrocyanide (King, 1946. *Micro-Analysis in Medical Biochemistry*, Churchill, London).

Silver :
1. Gravimetrically as AgCl.
2. By titration with thiocyanate.

Nitrite : Application of Bratton-Marshall sulphonamide method, as used by Hoagland to determine nitrite.

Potassium : From titration of silver with thiocyanate. See original paper (Cumings, 1939) for rationale.

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THE ENUMERATION OF BLOOD PLATELETS

BY

H. S. BAAR

(RECEIVED FOR PUBLICATION, JANUARY 6, 1948)

The platelets of human blood can, with reference to their fragility, be divided into two groups. The first group comprises the smallest platelets with very high fragility described for the first time by Floessner (1922). In the second group are the medium-sized and large platelets which have a higher degree of resistance and show in normal individuals a typical curve of disintegration (Baar and Szekely, 1929). The existence of the first group was questioned by Hartmann (1932), who regarded the tiny platelets as fragments of normal blood cells. Steinmaurer (1932) was, however, able to demonstrate by a special method this type of platelet in stained films (compare also Juergens, 1934). Apart from these platelets of normal people, there are also pathological giant forms (found especially in thrombocytopenic purpura) and exceptionally highly resistant pathological micro-platelets. The latter form was seen by Baar (1934) for a short period in a case of essential thrombocytopenia.

Corresponding to the two main types of platelets the technical methods for their enumeration may be divided into two groups. Thus the methods of Floessner (1922), Boshammer (1926), Juergens (1934), Steinmaurer (1932), Cumings (1933), and Olef (1935) permit the enumeration of all platelets, and the number found in normal individuals by these methods is 700,000 to 900,000 per c.mm. All these methods, however, are very laborious and unsuitable for routine laboratory work. Moreover, up to date no pathological conditions are known in which only the number of the most fragile platelets is decreased or increased without corresponding changes in the other groups of platelets. The much simpler methods of the second group supply us, therefore, with sufficient information. These are either indirect, in which the number of platelets is calculated from their relation to red blood corpuscles in stained films (Fonio, 1912) or in wet preparations (Cramer and Bannerman, 1929; Damashek, 1932; Leitner, 1935; Ladewig, 1935); or direct, in which the platelets are counted in a counting chamber (Spitz, 1921; Langmeyer, 1918; Kristenson, 1924; Tocantins, 1937; Wintrobe, 1946). The enumeration of platelets in Giemsa-stained films has the advantage that the morphology of platelets can be studied at the same time; it is, however, too time-consuming for routine work. The counting chamber methods have, on the other hand, various drawbacks. In some (Gutstein, 1932; Lenggenhager, 1936; Tocantins, 1937; Wintrobe, 1946) the platelets are counted in the presence of red blood corpuscles, which necessitates a comparatively high dilution; in others, agglutination and disintegration of platelets are not sufficiently suppressed. The latter objection applies also to a method described by the present author (1928) which is a combination of the methods of Kristenson and of Langmeyer. Reliable results are obtained only if the sample is counted immediately after collection. In addition, the diluting fluid deteriorates rapidly in spite of the presence of mercuric chloride, and loses its haemolytic property. Attempts were therefore made to replace urea by another more stable haemolytic substance, and at the same time to inhibit the disintegration of platelets. Saponin was found suitable for the first purpose. It has high erythrocytolytic action even in the smallest concentrations. An alteration of platelets, however, can also be noticed. In high concentrations they become dissolved in a short time. In medium concentrations the red cells are dissolved immediately; at first the platelets remain preserved, but after some minutes they lose their high refraction of light, become shadow-like, and finally disappear. In a concentration of 0.025 g. per 100 ml. the erythrocytes are quickly haemolysed while the platelets remain practically unaffected. A simple substitution of saponin for urea in Kristenson's fluid proved, however, impracticable because of the formation of numerous precipitates in the presence of saponin, mercuric chloride, and brilliant cresyl blue. Some of these precipitates can easily be differentiated from platelets and removed by filtration. Others, however, are spherical, pale blue, highly refractile, and can easily be mistaken for platelets; and it was impossible to remove these precipitates by filtration. This difficulty was overcome by the use of formaldehyde as preservative and fixative. Formaldehyde has a "tanning" action on the cell membranes. It thus prevents the disintegration of platelets, but owing to the

same property it counteracts the erythrocytolytic effect of saponin.

Method

A concentration of saponin and formaldehyde was finally found in which the red blood corpuscles are promptly haemolysed without the platelets being affected.

Saponin (B.D.H.)	... ...	0.25 g.
Sodium citrate	... ...	3.5 g.
Concentrated solution of formaldehyde (40%)	... ...	1.0 ml.
Brilliant cresyl blue	... ...	0.1 g.
Distilled water	to	100.0 ml.

The solution must be filtered once after preparation and remains unchanged for many months. The above formula is valid for the B.D.H. saponin only. For other saponins the proper concentration of saponin and formaldehyde can easily be found empirically, bearing in mind the above-mentioned action of these two substances.

For the enumeration of platelets a white cell pipette is used. The counting fluid is first sucked up to the mark 0.6, followed by blood from a finger-prick up to the mark 1.0 and counting fluid again up to the mark 11. The pipette is vigorously shaken for about 3 minutes immediately after collection and again before the counting chamber is filled. The enumeration can be done either immediately after collection or even several hours later without a change in the result. The platelets are easily recognized in the counting chamber by their light refraction and faint blue colour. No red blood corpuscles should be seen in the chamber. A high-power dry objective is used. When counting, the fine adjustment of the microscope must be continuously moved with wide excursions because the platelets are evenly distributed throughout the whole depth of the chamber (0.1 mm.). In cases with normal numbers of platelets it is sufficient to count half a square millimetre, but with thrombocytopenia the whole chamber or even both sides of a Buerker chamber should be counted. The dilution is 1 in 25, and the number of platelets in one square millimetre has therefore to be multiplied by 1,000 and divided by 4. The white blood cells are well stained and can be enumerated at the same time. Heparinized blood cannot be used.

Although no known method for enumeration of blood platelets can claim to be ideal, the present method has proved to compare favourably with many others which have been tried, so far as quickness and reliability are concerned. It has now been used for many years by junior technicians at the Children's Hospital, Birmingham.

I wish to thank Prof. J. M. Smellie for his interest in this work.

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J. clin. Path. (1948), **1**, 177.

MODIFICATIONS OF THE WEINBACH METHOD FOR THE DETERMINATION OF SODIUM IN SERUM

BY

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(RECEIVED FOR PUBLICATION, FEBRUARY, 1948)

The Weinbach (1935) method, based on that of Barber and Kolthoff (1928, 1929), involves the precipitation of sodium as the triple salt uranyl zinc sodium acetate in the presence of alcohol, and subsequent titration of this salt with sodium hydroxide, using phenolphthalein as indicator. We have found the end-point of the titration to be unsatisfactory, and have preferred colorimetric methods for this part of the determination. The colorimetric procedure, described by McCance and Shipp (1931), which depends on the colour produced by the addition of potassium ferrocyanide to a solution of the uranyl zinc sodium acetate in the presence of a small quantity of acetic acid, was found reasonably satisfactory so long as we used visual colorimetry, and compared the solution to be determined with a standard solution similarly treated, at the same time. When, later, we began to use photo-electric methods, difficulties arose owing to the rather rapid changes which take place in the intensity of the colour produced by this method. Indeed, the adoption of modern methods of colour measurement which do not involve direct comparison of two solutions made up under similar conditions at the same time has brought to light greater degrees of instability of many coloured solutions than were at first recognized. King and Garner (1947), for instance, have drawn attention to the fading of the blue colour of the solution used in many blood-sugar methods—a phenomenon which was immediately evident to us when we abandoned the usual visual colorimetric method for evaluating these solutions.

The change which occurs in the sodium determination by the ferrocyanide method is indicated in the Table, which gives the readings obtained on the same solution at different time intervals after adding the ferrocyanide; the instrument used is the Spekker photo-electric absorptiometer.

TABLE

VARIATION OF SPEKKER READINGS WITH TIME, FOLLOWING ADDITION OF POTASSIUM FERROCYANIDE TO SOLUTION OF TRIPLE ACETATE IN PRESENCE OF ACETIC ACID

Time (minutes)	Spekker reading
3	0.359
8	0.367
13	0.377
18	0.388
23	0.406
28	0.427

In view of these changes we decided to examine the method described by Darnell and Walker (1940). In this method the precipitated uranyl zinc sodium acetate, after suitable washing, is dissolved in water and treated with 5 per cent sulphosalicylic acid solution and 10 per cent sodium acetate solution. A clear, bright yellow solution results which we found to be very stable, and the curve given by readings with the Spekker instrument with sodium solutions corresponding to serum sodium concentrations over the range likely to be found in human blood was a straight line. The method has been in use here for some time and has proved to be very satisfactory.

More recently Bradbury (1946) has published a useful simplification of the uranyl zinc acetate procedure. He measured the colour of the supernatant fluid after the precipitation of the triple acetate, and so determined the reduction of colour produced by the precipitation: this reduction of colour was proportional to the amount of sodium present in the solution examined. This modification effects a useful saving of labour compared with earlier procedures, and, moreover, it avoids possible losses, either mechanical or by solution in

washing fluid, which are associated with the washing process.

We have found it necessary to make certain alterations in the procedure recommended by Bradbury. We have found that, with the quantities of reagent and alcohol recommended by him, precipitation of the triple salt is by no means complete in the twenty to thirty minutes he specifies. We have increased the proportion of alcohol and generally varied the volumes of fluids used so as to get complete precipitation, and at the same time to have readings of the colour of the supernatant fluid which shall give the most convenient range with the Spekker instrument when applying the method to serum sodium determinations. Even with the increased proportion of alcohol we find it necessary to let the mixture stand for two hours in the refrigerator at approximately 3° C. to ensure complete precipitation. The readings we have obtained with sodium solutions corresponding to serum concentrations of 200 to 400 mg. per 100 ml. fall on a straight line.

The full details of the method we use are as follows:

Method

To 1 ml. of serum add 1 ml. of water and 1 ml. of 20 per cent trichloracetic acid solution. Mix and allow to stand for 10 minutes; filter. To 1 ml. of filtrate in a small conical flask add 2 ml. of the Weinbach uranyl zinc acetate reagent,[1] 3 ml. of absolute alcohol, and 2 ml. of water. Mix thoroughly; stopper the flask, and allow it to stand in the refrigerator for two hours. Centrifuge the contents of the flask and determine the colour of the supernatant fluid in the Spekker photo-electric absorptiometer, using the Ilford violet filter 601. Subtract the Spekker reading of this fluid from the reading obtained with a similar mixture except that 1 ml. of water replaces the 1 ml. of filtrate, and obtain the sodium content of the serum by reading off the difference on the curve obtained by plotting the differences obtained from a series of standard sodium solutions.

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[1] *Preparation of Weinbach's uranyl-zinc-acetate reagent. Solution A*: 77 g. of uranyl acetate and 14 ml. of glacial acetic acid are dissolved with gentle heating and stirring in 400 ml. of water, and volume made up to 500 ml. in a volumetric flask. *Solution B*: 231 g. of zinc acetate and 7 ml. of glacial acetic acid are dissolved by gentle heating and stirring in 400 ml. of water, and volume made up to 500 ml. in a volumetric flask. The two solutions are mixed while hot, allowed to stand twenty-four hours or longer, and filtered.

INTERNATIONAL SOCIETY OF HEMATOLOGY

The International Society of Hematology will hold its biannual meeting at the Hotel Statler, in Buffalo, New York, from August 23 to 26, 1948. The following time has been tentatively allotted for symposia and presentations: half a day on general subjects, including radioactive and stable isotopes in haematology, half a day for problems and diseases related to the red cells, half a day for problems and diseases related to white cells, one day for immuno-haematology, Rh-Hr (CDE-cde) antigens and antibodies and haemolytic anaemias, half a day for coagulation problems and haemorrhagic diseases, and half a day for the business meeting.

Applications for the presentations of scientific exhibits are now being received by Dr. O. P. Jones, Department of Anatomy, University of Buffalo, Buffalo, New York. Chairman of the Programme Committee is Dr. Ernest Witebsky, Buffalo General Hospital, Buffalo, New York.

Dr. Eduardo Uribe Guerolo, Leibnitz 212, Nueva Colonia Anzurez, Mexico, D.F., is in charge of the programme from South and Central America, and Sir Lionel Whitby, University of Cambridge, England, is in charge of arrangements for the programme from Europe. Communications concerning applications for the programme will be received by the above-named committee men.

All scientific sessions and exhibits will be open to scientists interested in haematology. This will, of course, include members of the medical profession and those branches of science dealing with haematology, such as biochemistry, biophysics, genetics, immunology, etc.

Communications from interested haematologists in the United Kingdom, and applications concerning membership, should be sent to Dr. Robert R. Race, Lister Institute, Chelsea Bridge Road, London, S.W.1.

REVIEWS

The Background of Therapeutics. By J. Harold Burn. Geoffrey Cumberlege, Oxford University Press. 1948. Pp. 367. Price 22s. 6d.

This is not merely another book on therapeutics, but an attempt to describe the scientific background to recent therapeutic advances. The author is therefore frequently involved in discussions on bacteriology, as in the excellent review on methods of sterilization, and on chemistry, biochemistry, and pathological processes; all of which are in a clear style which is simple and easy to follow.

The author, in the preface, states that the work was undertaken after impressions, gained in America, of the importance placed in that country on laboratory and experimental work, and their value in improving diagnosis and treatment. He has used mainly British work as his basis, and has provided many British references in the hope that "Americans may learn of some of the work done in this country which ought to be more widely known." His approach to clinical research, which shows a full understanding of laboratory methods, will be found very acceptable to pathologists. It is clearly impossible to cover all the modern views on many varied subjects in 340 pages, but the condensation is on the whole admirably done. There are, however, several chapters which may well be recast in a future edition. The order of chapters might with benefit be arranged, so that disinfection, for example, is not squeezed between thyroid and allergy. The compound chapters are not all suitably chosen; for example a chapter dealing with plasma proteins and their place in cirrhosis of the liver ends up with the treatment of heart failure with digitalis.

There is an excellent half-chapter on iron metabolism, and it is hoped that in a new edition this will become the basis of a discussion on the therapeutics of the anaemias, instead of ending with a short discourse on calcium metabolism. The outstanding omission is, in fact, the absence of a review on blood and blood products in therapeutics, particularly as so much work on these subjects has been done in this country.

Clinical pathologists will be delighted to find, in a book written for clinicians, so many of the recent advances in investigational and experimental pathology which will give their colleagues an appreciation of the newer trends involving laboratory investigations, many of which will in the near future be in everyday demand in the control of modern therapeutic measures.

A. GORDON SIGNY.

Catalogue of Medical Films. Compiled by the Royal Society of Medicine and the Scientific Film Association. Published by ASLIB, 1948. Price 7s. 6d.

Those of us who know anything of the history of this jointly compiled catalogue have impatiently awaited its appearance. Now that it is available many will appreciate it and will realize that a document of this nature has been much needed in the past.

In this booklet are to be found the titles of some eight hundred films of medical interest, and although a good deal of space is devoted to an elaborate cross-indexing of these titles the net result is that films may be located numerically, generically, or alphabetically. In addition to this, details are given of some two hundred of these films: such details, however, are descriptive and not critical. This is perhaps disappointing, but the difficulties in the way of adequate appraisal must be considerable. It seems that we have not yet reached American standards in this respect; the American College of Surgeons have already laid down some criteria of medical film appraisal. Critical review, however, sometimes discourages the previewing of films by the teacher himself: this in turn leads to misuse and then to discredit.

The majority of those who refer to this work will be amazed at the wide choice of subject material, and it will now seem as easy to arrange a programme of films for an informal meeting as to select a single available film for the illustration of a specific lecture.

R. J. V. PULVERTAFT.

ASSOCIATION OF CLINICAL PATHOLOGISTS

COMPETITION FOR THE DESIGN OF A PATHOLOGICAL DEPARTMENT

The Association invites entries from interested individuals (e.g., pathologists, technicians, architects, and others) for this competition. Entries by two or more competitors jointly, will be permitted. The essay should include a description and plans for a department of pathology to serve a population of 200,000 to 300,000, and providing: clinical pathology for an "area" or "group" hospital and for general practitioners of the area, including domiciliary service; bacteriology for authorities in public health.

The following subjects and aspects of work should be surveyed: clerical and record services; amenities for patients and staff; bacteriology; biochemistry; haematology; morbid histology; morbid anatomy (including coroner's work and comprising post-mortem room and mortuary, not necessarily within the same building as the rest of the department). The competitor should describe and give detailed plans of type of building and layout of accommodation and should consider light, heat, ventilation, other systemic services, and the attributes and type of material used in building and in internal fixtures and fittings.

There will be first, second, and third prizes of respective values £50, £30, and £20. The competition will remain open until May 1, 1949, by which date all entries should have beent sent to the Hon. Secretary, Association of Clinical Pathologists, The Royal Infirmary, Worcester. The plan will remain the property of the competitor, but a copy of the plan and the accompanying essay will remain with the Association.

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ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.*

BACTERIOLOGY

"Aerosporin": an Antibiotic Produced by *Bacillus aerosporus* Greer. AINSWORTH, G. C., BROWN, A. M., and BROWNLEE, G. (1947). *Nature, Lond.*, **160**, 263.

An antibiotic for which the name "aerosporin" is proposed has been isolated from a Gram-positive spore-forming rod identical with that isolated by Greer from Chicago tap water and called *Bacillus aerosporus*. The organism has been isolated both from Surrey and Yorkshire soil, and can be extracted by methods similar to those used for streptomycin. It shows selective action against Gram-negative organisms, particularly *Bacterium coli*, *Haemophilus pertussis*, and *Salmonella typhi*, as well as against *Brucella bronchiseptica*. It is stated to be bactericidal in action, and weight for weight to have the same order of activity against Gram-negative as penicillin against Gram-positive organisms. Resistant strains could not be obtained by culture methods. *R. Wien.*

Polymyxin: A New Chemotherapeutic Agent. STANSLY, P. G., SHEPHERD, R. G., and WHITE H. J. (1947). *Bull. Johns Hopk. Hosp.*, **81**, 43.

This antibiotic, specific for Gram-negative bacteria, is produced in shallow or deep culture by the soil organism *Bacillus polymyxa*.

Streptomycin Therapy in Urinary Tract Infections. PULASKI, E. J., and AMSPACHER, W. H. (1947). *Surg. Gynec. Obstet.*, **85**, 107.

Of 465 patients with urinary-tract infections treated by streptomycin, 45% were not improved; calculi, pathological affections of the kidney (especially hydronephrosis, and paraplegia accounted for many of the failures. *Bacterium coli* and *B. aerogenes* infections responded well and *Pseudomonas* badly.

Guy Blackburn.

A Streptomycin Sensitivity Test. MATHEWS, A. G., and SHELTON, M. L. (1947). *Med. J. Aust.*, **2**, 387.

The authors investigate conditions, such as the suitability of the medium, effect of *p*H, and size of inoculum, affecting the estimation of the sensitiveness of bacteria to streptomycin, and describe a method giving reproducible results.

A Method for Determining Sensitivity to Penicillin and Streptomycin. HOYT, R. E., and LEVINE, M. G. (1947). *Science*, **106**, 171.

The authors have devised a method of sensitivity determination which obviates the use of a sterile technique and employs compressed tablets of penicillin and of streptomycin weighing 60 mg. each. The penicillin tablets contain approximately 1, 0.5, and 0.1 Oxford unit, and the streptomycin tablets contain 1, 0.1, and 0.01 mg. respectively. The agar plate is heavily streaked with the organism being tested, and the tablets are dropped into position with forceps. Such material as sputum, exudates, and infected body fluids can be tested directly without preliminary subculture. The plates are incubated, and are read when growth of the organisms is sufficiently advanced to show sensitivity or resistance.

R. Wien.

The Sensitivity of Organisms of the Genus *Leptospira* to Penicillin and Streptomycin. WYLIE, J. A. H., and VINCENT, E. (1947). *J. Path. Bact.*, **59**, 247.

Tests were made *in vitro* of the sensitivity to penicillin and streptomycin of 6 strains of *Leptospira icterohaemorrhagiae* and single strains of 23 other species of leptospira of European and Oriental origins. Streptomycin is stable but penicillin deteriorates on incubation, so the potency of the penicillin was estimated at the end of the experiment and it was found to have diminished by one-quarter to one-third of its original strength. Growth was markedly diminished in all cases and a number of strains were completely inhibited. In most cases penicillin was effective in a lower concentration than streptomycin.

The Relation of Protein Binding to the Pharmacology and Antibacterial Activity of Penicillins X, G, Dihydro F, and K. TOMPSETT, R., SCHULTZ, S., and MCDERMOTT, W. (1947). *J. Bact.*, **53**, 581.

Among these four penicillins the degrees of reduction in antibacterial activity caused by serum and albumin were roughly in direct proportion to the degrees of binding to these substances as demonstrated by dialysis. "The most satisfactory rationalization of these data is that the individual penicillins bind *in vitro* to a varying degree to the albumin content of serum, and that the resulting penicillin-albumin complex has little or no antibacterial activity."

The authors consider other possible explanations of their findings; examine evidence to suggest that binding of penicillin to protein occurs also *in vivo*; and say that their finding that penicillin sensitivity is different in different concentrations of serum may have an important bearing on the usual methods of assay of penicillin in serum.

A Study of the Absorption and Excretion of Oral Penicillin in Children. MARKOWITZ, M., and KUTTNER, A. C. (1947). *J. Pediat.*, **31**, 195.

It has been shown that gastric acidity is probably not a major factor in the destruction of orally administered penicillin and that the presence of food in the stomach influences penicillin absorption. The authors report their study on the absorption and excretion of oral penicillin as part of an investigation to determine its value as a measure for the prevention of streptococcal upper respiratory infections in rheumatic subjects, and conclude that oral administration of penicillin is a satisfactory procedure in children provided the drug is given under fasting conditions. *A. G. Watkins.*

The Effectiveness of Metachloridine in Suppressing Natural Infections with *Plasmodium malariae* and *P. falciparum* in British Guiana. KENNEY, K., and BRACKETT, S. (1947). *Amer. J. trop. Med.*, **27**, 493.

The suppressive effect of metachloridine on infections with *P. malariae* and *P. falciparum* was tested in schoolchildren of a malarious district of British Guiana; infections with *P. vivax* were too scarce to be included. *P. malariae* was found in only one blood film in the treated group, while 35 controls showed the presence of parasites. Fifty days after the end of treatment, however, there were 10 infections in the treated group and 23 in the controls. The effect on *P. falciparum* was much less, and it was necessary to double the dose. *P. falciparum* was found in 20 blood films of the first group and in 33 of the controls, and in 17 treated with the double dose compared with 60 controls. No toxic effects of metachloridine were seen in any of the trials.

***Streptobacillus moniliformis* Bacteremia with Minor Clinical Manifestations.** LEVINE, B., and CIVIN, W. H. (1947). *Arch. intern. Med.*, **80**, 53.

A case of rat-bite fever caused by *Streptobacillus moniliformis* is described. The clinical phenomena include an incubation period of a week, fever, fleeting arthralgia, a rash, and very little local inflammation. Penicillin is curative. *G. F. Walker.*

Cultivation of Tubercle Bacilli from Gastric Juice. A Study of the Factors affecting the Cultivation of *Mycobacterium tuberculosis* from Gastric Juice. VINCENT, V., and BIRGE, E. A. (1947). *Amer. Rev. Tuberc.*, **55**, 556.

The authors show that the survival of the tubercle bacillus depends on the acidity of the gastric juice: the more acid the specimen the shorter the survival time. To eliminate false negative reports gastric specimens should be examined immediately. When this is not possible, neutralization of the sample by the addition of sterile N/10 NaOH until the *p*H is 6.5 to 7.0 will allow the tubercle bacillus to live for several days.
R. J. Lumsden.

Aetiology and Pathogenesis of Vincent's Angina and of Ulcero-membranous Stomatitis. (Zur Aetiologie und Pathogenese der Vincent'schen Angina und der Stomatitis ulceromembranosa.) MELCZER, N. (1947). *Dermatologica*, **94**, 13.

The author demonstrated elementary bodies in smears from 26 cases of Vincent's angina. Animal inoculation with material from the lesions was negative, but 70% of the human cases reacted to intradermal injections. Bacteria-free filtrate of exudate mixed with bacterial suspensions caused phagedena-like ulcers. It is concluded that virus plus bacteria are responsible in symbiosis in the lesions.

Pathogenic Mechanism of Infective Phlebitis and the Genesis of Septicaemia. (Le mécanisme pathogénique des phlébites infectieuses et la genèse des septicémies.) REILLY, J., and GRISLAIN, J.-R. (1947). *Ann. Med.*, **48**, 113.

The authors, struck by the failure to produce phlebitis experimentally by the intravenous injection of organisms, decided to use the approach from the adventitia. They conclude that bacterial toxins and various soluble products of tissue breakdown can reach a vein from without, alter the endothelium, and so lead to thrombus formation. Any organisms circulating in the blood are entrapped in this thrombus. Thus an intravenous dose of organisms which in the healthy animal would be without apparent effect leads to infection of the thrombus and proliferation therein. The subsequent changes depend on the proteolytic activity of the organism used; if this is slight, further thrombosis occludes the infected portion, and after a brief bacteriaemia the blood remains sterile; if the organisms cause disintegration of the thrombus, then the progression is to pyaemia and fatal septicaemia. *A. C. Lendrum.*

Factors Influencing the Red Cell Agglutination-inhibitive Reaction in Influenza and their Application to the Diagnostic Test. WHITMAN, L. (1947). *J. Immunol.*, **56**, 167.

Though a considerable number of modifications have been made to the original red-cell agglutination test for influenza, the author considers that a need exists for a standard procedure simple enough to permit its widespread employment. He describes such a procedure. The PR 8 strain of influenza A virus and the Lee strain of influenza B virus were used in all experiments.
F. O. MacCallum.

Experience with Vaccination Against Influenza in the Spring of 1947. A Preliminary Report. FRANCIS, T., SALK, J. E., and QUILLIGAN, J. J. (1947). *Amer. J. publ. Hlth*, **37**, 1013.

Anticipating an outbreak of type-A influenza in the winter of 1946–7, the authors vaccinated 10,328 students with a vaccine having a red-cell agglutinating titre of 5120. 7615 students acted as controls. Excellent antibody responses were obtained, but investigations of the febrile illnesses showed no significant differences in the incidence of respiratory disease between the vaccinated and unvaccinated groups.

Early Immunization against Pertussis. ADAMS, J. M., KIMBALL, A. C., and ADAMS, F. H. (1947). *Amer. J. Dis. Child.*, **74**, 10.

The authors conclude that pertussis immunization in the first six months of life is a safe, practical, and desirable procedure. Reactions to inoculation were negligible. The weekly inoculations gave rapid agglutination response. It remains to be seen whether the titre level can be maintained by giving antigen at monthly intervals during the early months of infancy.

Aetiology of Typhoid Osteitis in Children Confirmed by Bone-marrow Culture. (Etiologia de las osteitis tificas en el nine comprobada por el mielo-cultivo.) JAUREGUY, M. A., and SIMON, G. (1947). *Arch. Pediat. Uruguay*, **18**, 298.

The authors found bone-marrow culture for diagnosis of typhoid fever so useful that they performed a Widal test, blood culture, and marrow culture as a routine. Out of 162 cases examined, 9 remained with persistently positive bone-marrow cultures.

Studies in the Pathogenesis of Rheumatic Fever. The Antistreptolysin Titre in Acute Tonsillitis and Rheumatic Fever. (In English.) WINBLAD, S., MALMROS, H., and WILANDER, O. (1947). *Acta med. scand.*, **196**, 533.

The investigation of antistreptolysin titre of 71 cases of acute tonsillitis was made serially using Ipsen's modification of Kalbak's method. Readings of over 200 were considered to be raised, and 52 cases showed some such elevation. Of the 14 who developed rheumatic complications, 12 had an initial titre of 140, and 9 a titre of 200 or more, suggesting previous infections with haemolytic streptococci.

Smear and Culture Diagnosis in Gonorrhoea. MCLEOD, J. W. (1947). *Brit. J. vener. Dis.*, **23**, 53.

In a series of 275 cases of suspected gonorrhoea in which 50% of the combined smear and culture results were positive, 42% of the cultures were positive against 29% of the smears; the cultures from the cervix and urethra were both positive in 22%, whereas only 7% of smears were positive from both these sites; from the urethra alone, the cultures were positive in 32% against 24% of positive smears; and the cervical cultures alone were positive in 33% against 16% of positive cervical smears. These figures show that diagnosis by culture is superior to that by smears and that the percentage of error is lessened when both methods are used together.

The author draws attention to Stuart's method (*Glasgow med. J.*, 1946, **27**, 131) of keeping gonococci under conditions of reduction and so preventing the oxidation which, with drying, is lethal to them.

T. Anwyl-Davies.

The Laboratory Diagnosis of Leprosy. MCDONALD, S. (1947). *Trans. R. Soc. trop. Med.*, **40**, 451.

Laboratory methods of confirming a clinical diagnosis of leprosy comprise: (*a*) the examination of skin smears stained by the Ziehl-Neelsen method, which is especially suitable for lepromatous and major tuberculoid forms; (*b*) the lepromin test; (*c*) Wassermann and Kahn tests; (*d*) skin biopsy.

BIOCHEMISTRY

Insulin Resistance. AXELROD, A. R., LOBE, S., ORTEN J. M., and MYERS, G. B. (1947). *Ann. intern. Med.*, **27**, 555.

Insulin resistance has occurred in conditions favouring the destruction of insulin in the body, particularly associated with abnormal leucocyte activity. Investigating 3 cases of spontaneous insulin resistance, the authors found no evidence of an insulin antagonist in the sera of their patients. Whilst the aetiology remains unknown no specific therapeutic measures are available. Single large doses are recommended in treatment, rather than repeated smaller doses, and glycosuria and acidosis may require as much as 1,000 to 5,000 units of insulin.

Serum Potassium, Magnesium, and Calcium Levels in Diabetic Acidosis. MARTIN, H. E., and WERTMAN, M. (1947). *J. clin. Invest.*, **26**, 217.

The changes in the levels of serum potassium, magnesium, and calcium were studied in 14 patients, aged 12 to 69 years, undergoing treatment for severe diabetic acidosis. The blood-sugar levels varied between 285 and 1,029 mg. per 100 ml. and the insulin requirement in the first 24 hours between 110 and 1,000 units; large amounts of fluid (4 to 12 litres) were given parenterally in the first 24 hours. There was a marked fall in the serum potassium in 46% of patients; critically low values (1.9 and 2.18 milliequivalents) were found in some. The minimum was usually reached after 12 to 24 hours. No constant correlation between total calcium concentration and ionized calcium was found. A marked fall in the serum-magnesium concentration was seen in 36% of the patients during treatment; concentration returned to normal very slowly. It is suggested that potassium and magnesium salts should be given as adjuvants to therapy, but this is contraindicated in patients in shock and with decreased renal function, because dangerously high blood levels may be produced.

A. Schott.

Carbohydrate Metabolism in the Undernourished. (Der Kohlehydratstoffwechsel bei Unterernährten.) GÜLZOW, M. (1947). *Z. ges. inn. Med.*, **2**, 91.

The first part of this paper is a study of 45 cases of inanition, mostly with oedema, 25 of which were fatal. Hypoglycaemia was marked and sugar-tolerance tests always gave a poor response with a flat curve and a very low and delayed peak. After about 1 month on an ample diet in hospital injections of adrenaline and insulin gave normal responses.

A further study was made in order to ascertain the site of the metabolic lesion in starvation, and the observed failure of the tissues to assimilate sugar is attributed to damage to cell enzymic processes and to diminution in hormonal action, especially insulin insufficiency.

The Effect of Ingested Mineral Oil on Plasma and Vitamin A. ALEXANDER, B., LORENZEN, E., HOFFMANN, R. and GARFINKEL, A. (1947). *Proc. Soc. exp. Biol., N.Y.* **65**, 275.

Continued ingestion of mineral oil as such or in the form of mayonnaise dressing by 20 subjects on a normal unrestricted diet resulted in a moderate decrease in plasma carotene concentration. Vitamin A levels remained unchanged. It is concluded that simultaneous ingestion

of mineral oil with food prevents substantial amounts of food carotene from entering the body.—[Authors' summary.]

A Study of Thiamine in Paediatrics. (Studie o thiaminu v pediatrii.) HOUŠTĚK, J. (1947). *Čas. Lék. čes.*, **86**, 1006.

Present methods of vitamin B_1 estimation are reviewed. Although physico-chemical methods with thiochrome are most often used, the author prefers Schopfer's microbiological technique because of its specificity and accuracy. It is based on the fact that *Phycomyces blakesleeanus* requires thiamine for growth but is unable to synthesize it.

Breast-milk seems the only source of thiamine for the infant. Synthesis of thiamine in the intestine is doubtful. Breast-milk of 233 mothers of all classes had a vitamin B_1 content of 15 to 19 μg. per 100 ml. Some differences were encountered in various age groups. Colostrum is free from vitamin B_1. The vitamin content increases from 13.8 μg. per 100 ml. in the first week of lactation to 20.2 μg. per 100 ml. after the second month. Saturation of the mother with 50 to 100 mg. thiamine by oral, intravenous, or intramuscular administration raises the level in breast-milk transiently. It is highest after intravenous injection. The blood level remains lower than the level in the milk.

The vitamin-B_1 content of the cerebrospinal fluid was estimated in 105 children of all ages with various affections of the nervous system. The values were fairly constant and ranged from 1 to 4 μg. per 100 ml. independent of age, condition, or cell and protein content of the liquor. The diffusion of thiamine into the spinal fluid was demonstrated. After intravenous doses of 50 mg. vitamin B_1 the level increased from 1 to 2.5 μg. to 12.5 to 16.5 μg. per 100 ml. With lower doses the increase was negligible.

The thiamine level in blood (usually capillary) was examined in 180 children. A level of 5 to 8 μg. per 100 ml. was found. Values below 4 μg. per 100 ml. are considered to reveal a latent hypovitaminosis. Premature infants had a relatively high level (mean value 9.8 μg. per 100 ml.). The estimation of thiamine in blood is considered unsatisfactory and inconclusive, even with Schopfer's method. The vitamin-B_1 equivalent is recorded as a whole, without distinction of free and fixed vitamin and of intermediate metabolites.

M. Dynski-Klein.

A Study of the Fasting-hour Excretion of Thiamine in the Urine of Normal Subjects. PAPAGEORGE, E., and LEWIS, G. T. (1947). *J. Nutrit.*, **34**, 301.

The authors have estimated the 24-hour urinary excretion of thiamine by 63 normal university students, and have compared the values with the amount in a "fasting-hour" specimen, collected in the hour following the 24-hour period and after an overnight fast of about 12 hours. There is a good correlation between the values. In 20 of their 63 subjects the authors also determined the percentage of a 1-mg. oral dose of thiamine excreted in the next 4 hours in the fasting state. The authors therefore confirm previous work which indicates that the fasting-hour excretion is a convenient method in nutritional surveys of obtaining an approximate indication of the trend of thiamine levels in the body. They suggest that the critical level of fasting-hour excretion of thiamine is 4 μg. *H. M. Sinclair.*

Factors Influencing the Urinary Excretion of Calcium. I. In Normal Persons. KNAPP, E. L. (1947). *J. clin. Invest.*, **26**, 182.

The normal urinary excretion of calcium was studied under standard dietary conditions in 606 normal persons ranging in age from 1 to 80 years. The mean urinary calcium excretion increased with both age and intake; in adults the increase in excretion with increase in intake is greater than in children. With constant daily calcium intake the mean urinary excretion increased with age throughout the period of growth. The skeletal weight was found to be the factor responsible for these differences as well as for those with sex. The urinary calcium values calculated as a percentage of calcium intake were plotted against the calcium intake per kilo of body weight; it was found that $\frac{\text{urinary Ca} \times 100}{\text{Ca intake}}$ was an exponential function of, and varied inversely with, the intake per kilo. The position of urinary calcium values in any individual person relative to the mean depended on the endocrine balance of the person and was independent of age or calcium intake. Normal infants, irrespective of a low or high calcium intake per kilo, had a level of urinary excretion within the range of normal for older subjects but below the mean for this latter group, the mean level characteristic for older subjects being reached at about the age of 2. Undernourished children had a uniformly low calcium excretion, which rose sharply as the child gained weight. Ingested acids (ammonium chloride, ketogenic diet) and a high calcium/phosphorus ratio, without altering the total calcium content of the diet, increased urinary calcium. Some other dietary factors of less importance in urinary calcium excretion are discussed.

A. Schott.

Metabolism of Women during the Reproductive Cycle. XIII. The Utilization of Niacin during Lactation. CORYELL, M., RODERUCK, C., HARRIS, M. E., MILLER, S., RUTLEDGE, M. M., WILLIAMS, H. H., and MACY, I. G. (1947). *J. Nutrit.*, **34**, 219.

Niacin intake, secretion in milk, and excretion in urine were studied in 7 healthy nursing mothers during the first 10 days post partum, and in 9 women during periods of 5 consecutive days 2 to 10 months post partum.

The average daily intakes of niacin and its precursor during the 5-day periods ranged from 13 to 23.4 mg. The results show during the first 10 days post partum rapid increases in the amounts of niacin secreted daily in milk, averaging from 0.04 mg. on the first day post partum to 2.9 mg. on the tenth; these figures portray the increases both in concentration and in volume of milk secreted during the puerperium. The average daily niacin content of mature milk ranged from 0.52 to 2.02 mg.; these values, also, show a general relationship to milk volume rather than to intake, and illustrate the wide range of normal variation in the composition of human milk from different mothers and from the same mother at different times. *J. Parness.*

Oral Smears Compared with Vaginal Smears. Case Report. RUSSELL, P. B. (1947). *Sth. med. J.*, **40**, 561.

This is a case report to illustrate the value of Russell and Bennett's method in controlling the synthetic oestrogen therapy of menopausal symptoms in a woman of 44, following surgical operation. Gram-stained smears from the surface of the mouth show the influence

of hormonal stimulation on the buccal epithelium, and permanent preparations can be made for comparison. It is suggested that the method is applicable to the oestrogen therapy of vulvo-vaginitis in children, and that the patients themselves could prepare the smears.

E. T. Ruston.

The Effect of Trauma and Disease on the Urinary 17-Ketosteroid Excretion in Man. FORBES, A. P., DONALDSON, E. C., REIFENSTEIN, E. C., and ALBRIGHT, F. (1947). *J. clin. Endocrinol.*, **7**, 264.

Analysis of 4,000 determinations of urinary 17-ketosteroid excretion showed that the output was low in chronically ill or seriously undernourished patients. Members of the laboratory staff whose basal output was well established showed a 70% reduction in output during acute infections. The effect of various types of "alarm," including operation, acute infections, etc., showed an immediate rise in excretion in men, followed by a period of subnormal excretion. Women showed no preliminary rise.

The Excretion of 17-Ketosteroids and Gonadotrophin in Children: Normal and Abnormal Cases. HAIN, A. M. (1947). *Arch. Dis. Childh.*, **22**, 152.

Gonadotrophin can be detected in normal girls before the seventh year and in normal boys before the ninth. It is excreted in abnormally large amounts in cases of precocious sexual development. Constitutional precocious menstruation is probably due to premature pituitary secretion in association with abnormal ovarian response. Instances of the use of hormone excretion assays in various types of endocrine and constitutional abnormalities are given. *P. R. Evans.*

Correlations of Biochemical and Histologic Changes in the Adrenal Cortex. ROGERS, W. F., and WILLIAMS, R. H. (1947). *Arch. Path.*, **44**, 126.

The adrenal cortex was studied in a number of healthy persons who died instantaneously after traumatic injury and in whom the glands could justifiably be regarded as normal. Comparisons were made between the cholesterol content and the amount of lipid present, as demonstrated in histological section by use of polarized light, ultraviolet light, and phenylhydrazine and Sudan IV stains in frozen sections. Phloxine-methylene-blue was used in paraffin sections to study vacuolation. The amount of lipid demonstrated by the first three methods was directly proportional to the cholesterol content. Similar correlation was found in glands depleted of lipid in severe toxic states such as fulminating streptococcal septicaemia and by administration of adrenotrophic pituitary hormone. *W. S. Killpack.*

Proteins in the Colloidal Gold Reaction. BERNSOHN, J., and BORMAN, E. K. (1947). *J. clin. Invest.*, **26**, 1026.

The authors suggest that abnormal colloidal gold reactions are due to a significant change in the γ-globulin content or to an alteration in the proportions of β- and possibly α-globulin to the γ-globulin. A spinal fluid giving the general paretic curve (type I) contains significantly less protective globulins and generally more γ-globulin. A spinal fluid giving the curve found in bacterial meningitides (type III) contains more globulin of the β type than a normal fluid. Other curves of type II can be explained on an alteration of the ratio of the protective to the γ-globulins. The reactions obtained with blood serum in cases of liver dysfunction arise from the relative decrease of protective globulins which are elaborated by the liver cells. The authors suggest that γ-globulin could be used to determine whether or not two batches of gold sol are comparable. *E. T. Ruston.*

A Microbiological Method of Standardizing Liver Extracts. MAHDIHASSAN, S., and BAKSHI, V. M. (1947). *Nature, Lond.*, **160**, 404.

A bacterium, *Bacterium carotene*, producing a red pigment was isolated from the insect *Cicadella viridis* and grown on artificial media containing liver extract. Growth occurred only when liver extract was added to the medium, and it appeared to be related quantitatively to the concentration of liver extract. The growth factor present in the liver extracts was found to resist autoclaving for 6 hours at 15-lb. pressure, and to be adsorbed on to fuller's earth and activated charcoal but not on to kaolin. It is suggested that this organism may provide a method of microbiological assay for standardizing liver extracts.

Hepatic Dysfunction in Peptic Ulcer. Observations with the Hippuric-acid Test. POLLAK, H. (1947). *Lancet*, **2**, 131.

Oral hippuric-acid excretion tests were performed on 68 male patients with peptic ulcer. No other tests of liver function are recorded and no details of the clinical condition of these patients are given, but the author concludes that they had a tendency to depressed liver function, the impairment being greater in the active phase of the disease. *C. Hardwick.*

Liver Function Tests in the Diagnosis of Jaundice. A Review of 200 Cases. MACLAGAN, N. F. (1947). *Brit. med. J.*, **2**, 197.

Serum alkaline phosphatase shows a maximum deviation from normal in obstructive jaundice, whereas the flocculation tests show maximum abnormality in hepatitis. The present paper is concerned with the results obtained in 200 jaundiced patients, in whom the cause of the jaundice was satisfactorily established by other means. Of these, 56 were cases of obstructive jaundice (37 due to neoplasm) and 118 of acute hepatitis (95 infective hepatitis), and there were also 15 cases of chronic hepatitis and 11 of haemolytic jaundice.

All cases with phosphatase levels above 42 units were obstructive. All with phosphatase levels below 15 units were non-obstructive, and this group included all cases of haemolytic jaundice. All cases with strongly positive flocculation reactions were non-obstructive; all those with weak or negative flocculation reactions and phosphatase levels above 35 units were obstructive.

Douglas H. Collins.

Chronic Liver Disease Following Infectious Hepatitis. I. Abnormal Convalescence from Initial Attack. KUNKEL, H. G., LABBY, D. H., and HOAGLAND, C. L. (1947). *Ann. intern. Med.*, **27**, 202.

Reviewing 350 cases of the disease, with a special study of tests of liver efficiency, the authors describe as necessary for the estimation of persistent impairment of the liver a combined use of determinations of the plasma bilirubin level, the bromsulphalein retention test, and the thymol turbidity reaction of the serum. Under an elaborate scheme of chemical testing the authors found

that 17% of their patients sustained relapses or other forms of delayed healing due to functional hepatic impairment; in several, recovery was delayed for over a year. *G. F. Walker.*

A Study of Hepatic Function in Acquired Malaria. SCHNEIDER, L. A., and SHALLENBERGER, P. L. (1947). *Amer. J. med. Sci.*, **214**, 80.

Approximately one-third of all acute acquired malaria cases under atebrin therapy are shown to have demonstrable hepatic dysfunction as evidenced by standard liver function test.

Changes in Blood Phosphate after Ingestion of Glucose and Fructose in Sprue. FOURMAN, L. P. R. (1947). *Brit. med. J.*, **2**, 411.

Inorganic phosphate concentrations in the plasma, cells, and whole blood after the ingestion of 50 g. of glucose and the ingestion of 50 g. of fructose were estimated in 5 normal subjects and in 4 patients suffering from tropical sprue. Changes in blood "ester" phosphate concentrations and in the rate of phosphate excretion in the urine were also observed.

In normal subjects the plasma phosphate diminished after the ingestion of both glucose and fructose, whereas in the sprue patients after glucose the drop in phosphate was less than in the normal, and after fructose the phosphate remained unchanged or rose. These findings are taken as evidence that in the sprue patients there was a failure of absorption of glucose and fructose.
H. K. Goadby.

Measurement of the Concentration of Miracil in Biological Fluids. LATNER, A. L., COXON, R. V., and KING, E. J. (1947). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 133.

"Miracil D" (l-diethylaminoethylamino-4-methylthioxanthone) has been shown by German workers to be active against schistosome infections in laboratory animals. In view of the possible use of the drug in medicine methods have been worked out for its determination in blood and urine. Blood concentrations may be determined by colorimetric measurement of the amount of bromothymol blue which will combine with the miracil extracted from blood with ethylene dichloride, or by determination of the yellow colour of the drug extracted from the sample. *L. G. Goodwin.*

The Value of Spinal Fluid Examination as a Diagnostic Procedure in Weil's Disease. CARGILL, W. H., and BEESON, P. B. (1947). *Ann. intern. Med.*, **27**, 396.

Of 14 cases of Weil's disease observed during a 4-year period, 13 were found to have abnormal spinal fluid. In only 6 were there clinical signs which could be attributed to meningeal irritation. The commonest abnormality was an increase in the cell count. Xanthochromia was noted in approximately 90% of the cases in which jaundice was present. Spinal fluid examination is of value as a routine diagnostic procedure when the diagnosis of Weil's disease is suspected.

On Changes in the Cerebrospinal Fluid during Measles. (In English.) OJALA, A. (1947). *Ann. Med. intern. fenn.*, **36**, 321.

After investigating 123 unselected cases of measles without clinical evidence of central nervous system involvement, and 4 cases in which lumbar puncture was called for on clinical grounds, the author concludes that pleocytosis in measles (nearly 30% in his series) is more frequent than has been previously recorded. Changes in the cerebrospinal fluid may be due to the virus itself or its toxins, and the view is expressed that in measles pleocytosis is an allergic phenomenon caused by toxin. The author believes that measles encephalitis is more common than is assumed but that the symptoms are chiefly psychic. *E. H. R. Harries.*

Caronamide for Increasing Penicillin Plasma Concentrations in Man. CROSSON, J. W., BOGER, W. P., SHAW, C. C., and MILLER, A. K. (1947). *J. Amer. med. Ass.*, **134**, 1528.

Normally, about 80% of the penicillin in the urine is excreted by the renal tubules and only about 20% by glomerular filtration. The excretion of the drug by the tubules can be completely suppressed by the intravenous administration of either iodopyrin ("diodrast") or *p*-aminohippuric acid, but the usefulness of this procedure is limited by the large amounts of either of these compounds which have to be injected.

This report describes the clinical application of caronamide (4′-carboxy-phenylmethanesulphonanilide), an orally effective compound of low toxicity capable of producing a reversible inhibition of penicillin excretion by the renal tubules. The effectiveness of caronamide depends upon a "substrate competition between penicillin, which is excreted by the tubules, and 4′-carboxyphenylmethanesulphonanilide, which is essentially refractory to excretion by that transport mechanism."

Of 6 patients studied, 5 showed increases in plasma penicillin concentrations during the period of administration of caronamide. The average concentrations during the period of administration of the new drug were from 2.4 to 7.1 times greater than during the two control periods. *A. L. Walpole.*

Oliguria after Abortion. HUMPHREY, J. H., and JONES, F. A. (1947). *Clin. Sci.*, **6**, 173.

Renal failure with oliguria after abortion (of which only 19 cases have been hitherto reported) may be due to pyelonephritis, ingested poisons, sulphonamides, quinine sensitivity, incompatible transfusion, a syndrome with renal changes resembling those of crush syndrome, or symmetrical cortical necrosis. Four patients, all of whom recovered with "conservative" treatment, are fully described. The pathogenesis of this syndrome is discussed in the light of the work of Barclay and others (*Lancet*, 1946, **2**, 237). The pathological process involved was thought to be renal vessel spasm with or without thrombosis, such as is found in persons dying with symmetrical renal cortical necrosis.

Studies in Urolithiasis : I. The Composition of Urinary Calculi. PRIEN, E. L., and FRONDEL, C. (1947). *J. Urol.*, **57**, 949.

Approximately 700 urinary calculi were studied under the polarizing microscope. Only 9 distinct crystalline substances were found, and these fell into the following groups: oxalates, phosphates, uric acid, urates, and cystine. Detailed descriptions and chemical analyses are given of the stone formations. As many as 4 crystalline compounds were often found by optical analysis in the same calculus.

HAEMATOLOGY

Does Rh-Isoimmunization Cause Early Abortion? OVERSTREET, E. W., TRAUT, H. F., HUNT, M., and LUCIA, S. P. (1947). *Amer. J. Obstet. Gynec.*, **54**, 235.

The authors conclude that there is no evidence that Rh-iso-immunization may cause abortion in the first 20 weeks of pregnancy.

The Role of a Presumed Serum Protein in the Pathogenesis of Erythroblastosis Fetalis. GUREVITCH, J., POLISHUK, Z., and HERMONI, D. (1947). *Amer. J. clin. Path.*, **17**, 465.

The authors conclude that sera from umbilical cords and from infants less than 6 months of age do not enhance the agglutinating action of immune antibodies as do normal adult sera.

Use of Trypsin in the Detection of Incomplete Anti-Rh Antibodies. MORTON, J. A., and PICKLES, M. M. (1947). *Nature, Lond.*, **159**, 779.

Rh-positive cells sensitized *in vitro* with Anti-D incomplete antibody, and cells from infants suffering from haemolytic disease of the newborn were agglutinated by solutions of trypsin.

Kernicterus. A Follow-up Study of Thirty-five Erythroblastotic Infants. STILLER, R. (1947). *Amer. J. Dis. Child.*, 73, 651.

Out of 35 children born with erythroblastosis, 29 survived; 4 showed signs of C.N.S. involvement, 2 of these in the neonatal period. Transfusion is of no value in ameliorating damage to the brain.

Modification of Human Red Cells by Virus Action. CHU, C. M., and COOMBS, R. R. A. (1947). *Lancet*, **1**, 484.

After treatment with the haemagglutinating viruses of influenza A and B, swine influenza, and Newcastle disease (N.D.V.), Rh-positive cells were agglutinated by incomplete Rh antibody. The order of activity in this respect was as follows: N.D.V.>swine influenza> influenza B>influenza A. Rh-negative cells were not agglutinated.

The Improved Demonstration of Circulating Antibodies in Hemolytic Anemia by the Use of a Bovine Albumin Medium. NEBER, J., and DAMESHEK, W. (1947). *Blood*, **2**, 371.

Using 20% albumin as diluting fluid, the authors claim to have demonstrated "warm agglutinins" in 5 patients with acquired haemolytic anaemia; in 4 of these no agglutinin was demonstrable when saline was used instead of the albumin. Employing the albumin technique they demonstrated a serum haemolysin in one patient with acquired haemolytic anaemia and in a case of familiar spherocytic anaemia during a haemolytic crisis.

The Significance of the van den Bergh Reaction. GRAY, C. H. (1947). *Quart. J. Med.*, **16**, 135.

The author claims that the terms "prompt," "biphasic," and "delayed" reactions are obsolete, as they depend on the amount of bilirubin present and not on the type of jaundice. The direct-indirect quotient (D.I.Q.), i.e.,

$$\frac{\text{final amount of azobilirubin in the direct reaction}}{\text{final amount of azobilirubin in the indirect reaction}} \times 100$$

is a useful calculation. Below 40, this is diagnostic of haemolytic jaundice; above 50, it indicates obstructive or hepatogenous jaundice. The van den Bergh reaction is quite incapable of differentiating obstructive from hepatogenous jaundice.

Biochemical and Physiological Studies of Two Active Substances Produced by the Spleen. (Études biochimiques et physiologiques sur deux substances actives produites par la rate.) UNGAR, G. (1947). *J. Physiol., Paris*, **39**, 219.

Two physiologically active substances, splenin A and B (*Endocrinology*, 1945, **37**, 329), have been isolated from ox, horse, and sheep spleen and have been produced by an *in vitro* method. Splenin A decreases and splenin B increases bleeding time. Thus, in guinea-pigs one unit of splenin A per kilo injected subcutaneously lowered the mean bleeding time from 131 to 100 seconds, and one unit of splenin B raised it from 131 to 160 seconds.

Splenin A is normally found in the spleen (about 10,000 units per g.), from which it flows into the blood stream and is excreted in the urine. Splenin B occurs in the spleen (about 30 units per g.) and bone marrow (about 30,000 units per g.), but is not normally found in the blood stream. Splenin B is probably identical with thrombocytopen (Troland and Lee, *J. Amer. med. Ass.*, 1938, **111**, 221). Splenin A and B have opposing pharmacological actions on bleeding time, capillary permeability, and haemolysis, the former promoting and the latter retarding combination between the protease and anti-protease of blood. When excess splenin B is produced, it passes into the blood stream and interferes with the mechanism of certain pathological states such as purpura and haemolytic jaundice. Splenin A, the liberation of which may be controlled by the hypophysis and suprarenal cortex, intervenes during the adaptation syndrome probably by neutralizing protease and thus protecting the capillary endothelium. Its pharmacological properties are similar to those of vitamin P substances such as hesperidin and rustin. *J. E. Page.*

Studies on the Enigma of the Hemostatic Dysfunction of Haemophilia. QUICK, A. J. (1947). *Amer. J. med. Sci.*, **214**, 272.

The author describes experiments which in his opinion show that the defect of coagulation in haemophilia is due to lack of thromboplastinogen, the inactive precursor of thromboplastin. Other components are present in normal proportions.

Some Clinical Observations on the Prothrombin Test. FISHER, B. (1947). *Amer. J. clin. Path.*, **17**, 471.

Whole plasma is recommended for use in Quick's one-stage method for the estimation of prothrombin. Dilution with saline, rather than with prothrombin-free plasma, introduces errors. Prothrombin levels should

$$\left(= \frac{302}{\text{prothrombin time in sec.}-8.7}\right)$$

also be expressed as percentages of prothrombin (Quick), and not in seconds or as percentages of prothrombin times determined in normal subjects.

Significance of the Accelerated Reaction in Determination of Prothrombin Time of Diluted Plasma. TUFT, H. S., and ROSENFIELD, R. E. (1947). *Amer. J. clin. Path.*, **17**, 704.

A 10% dilution of plasma employed in 500 deter-

minations did not indicate that a decreased prothrombin time was correlated with clinical evidence of thrombosis or embolism.

Variations in Prothrombin and Antithrombin in Patients with Thrombosing Tendencies. HURN, M., BARKER, N. W., and MANN, F. D. (1947). *Amer. J. clin. Path.*, **17**, 709.

The plasma prothrombin by the one- or two-stage methods and serum antithrombin levels were estimated in 63 patients with thrombotic or embolic conditions. There was no diagnostic correlation between clinical state and laboratory observation.

Variations in Prothrombin and Antithrombin Following the Administration of Dicoumarol. HURN, M., BARKER, N. W., and MANN, F. D. (1947). *Amer. J. clin. Path.*, **17**, 712.

The authors compare the one- and two-stage techniques for estimating prothrombin times. The one-stage technique, which depends upon the rate of conversion of prothrombin, is held to be preferable in the control of dicoumarol therapy.

Congenital Hypoprothrombinaemia and Pseudo-hypoprothrombinaemia. QUICK, A. J. (1947). *Lancet*, **2**, 379.

The author summarizes his views on blood coagulation, and describes a labile factor (probably identical with Owren's factor 5) whose disappearance causes an increase in prothrombin time when plasma is stored. Two families are described; one suffering from true familial hypoprothrombinaemia; the other from "pseudo-hypoprothrombinaemia." In the latter the prothrombin time was elevated, but prothrombin and the labile factor were present in normal preparations. The lack of still another factor is postulated.

Essential Thrombocytopenic Purpura. Autopsy Findings in Thirty-six Cases. HERTZOG, A. J. (1947). *J. Lab. clin. Med.*, **32**, 618.

The post-mortem findings in 36 cases of essential thrombocytopenic purpura are described; these are taken from the records of over 51,600 necropsies performed at the University of Minnesota. Haemorrhages were the chief necropsy findings; in 12 cases intracranial haemorrhages caused death. In 2 cases bleeding from the gastro-intestinal tract predominated. In 1 the principal bleeding was from the renal pelvis and bladder. Nine patients had continued to suffer from widespread haemorrhages after splenectomy. There were no characteristic histological findings. The bone marrow was examined in 10 of the 36 cases. The most constant feature was a marked increase in the number of megakaryocytes. The author suggests that, where the marrow shows a marked decrease in number of megakaryocytes, little or no benefit is derived from splenectomy.

R. Winston Evans.

Influence of Penicillin on the Coagulation of Blood, with Especial Reference to Certain Dental Operations. FLEMING, A., and FISH, E. W. (1947). *Brit. med. J.*, **2**, 242.

With high concentrations of penicillin (1,000 units per ml. or more) there is some interference with the clotting mechanism. This effect is of no practical importance in the systemic administration of penicillin. It should be borne in mind when employing solutions of high concentration locally.

Leucocytosis and Changes in the Differential White Cell Count in Renal Colic. (Sul comportamento della leucocitosi e della formula leucocitaria nella colica renale.) BELUFFI, E. L., and FANTONI, S. (1947). *Ann. ital. chir.*, **24**, 152.

Cases are cited of right-sided pain due to uncomplicated non-infected ureteric calculi which are associated with leucocytosis up to 14,000 per c.mm. Doubt is thus thrown on the value of the leucocyte count in differentiating appendicitis from ureteric colic.

Idiopathic Thrombocytopenic Purpura. A Study of Three Cases with Special Reference to Changes in the Megakaryocytes. VALENTINE, E. H. (1947). *Amer. J. med. Sci.*, **214**, 260.

A decreased formation of platelets by an increased number of megakaryocytes is held to be due to an inhibiting factor secreted by the spleen.

Iron-staining Erythrocytic Inclusions with Especial Reference to Acquired Haemolytic Anaemia. MCFADZEAN, A. J. S., and DAVIS, L. J. (1947). *Glasg. med. J.*, **28**, 237.

The authors confirm and extend the observations of Pappenheimer *et al.* (*Quart. J. Med.*, 1945, n.s., **14**, 75), who described the appearance of numerous stippled red cells in patients with acquired haemolytic anaemia after splenectomy. The basophilic inclusions are found in both nucleated and adult red cells. They react with acid-potassium ferrocyanide and are believed to correspond with the siderotic granules of Gruneberg. Their formation is attributed to some abnormality in haemoglobin formation.

Development of Inclusion Bodies containing Ribose Nucleic Acid in Myeloma Cells after Injections of Stilbamidine. Determination of Stilbamidine in Myeloma Tissue. SNAPPER, I., MIRSKY, A. E., SCHNEID, B., and ROSENTHAL, M. (1947). *Blood*, **2**, 311.

Basophilic granules containing ribose nucleic acid not normally present in the cytoplasm of myeloma cells appear after injections of stilbamidine. Methods are given for identifying ribose nucleic acid, and for the estimation of stilbamidine in myeloma tissue.

The Diffraction Method of Measuring Red Blood Cells. PIJPER, A. (1947). *J. Lab. clin. Med.*, **32**, 857.

The author reviews the development of the diffraction technique of measuring red-cell diameters and describes an improved apparatus whereby it is possible to calculate the degree and quality of anisocytosis, as well as mean cell diameter.

The Osmotic Resistance (Fragility) of Human Red Cells. PARPART, A. K., LORENZ, P. B., PARPART, E. R., GREGG, J. R., and CHASE, A. M. (1947). *J. clin. Invest.*, **26**, 636.

Temperature, *p*H, and time as well as salt concentration determine the apparent fragility of red blood cells. This paper should be read by all interested in this determination.

MORBID ANATOMY AND HISTOLOGY

Adnexal Carcinoma of the Skin. CHANDLER, N. (1947). *Amer. J. Path.*, **23**, 1.

This paper gives an account of the group of "basal-cell carcinomata," or, as the author rightly prefers to call them, "adnexal carcinomata of the skin." The present position as regards nomenclature, classification, and histogenesis of these growths is confused, and the author bases his attempt to bring order out of the existing chaos on a careful study of over 200 tumours. The interesting and fully documented historical review reveals little that is essentially new.

The subdivisions given are as follows: (1) *Pilar Type*; (2) *Sudoriparous Glandular Type*; (3) *Basal-celled Type*.

The author's theory, based on his observations and on a consideration of the development of epidermal structures, is that adnexal carcinomata are derived not from basal cells of the epidermis but from the hair follicles, sweat glands, or rather from cells of the embryonal rudiments of these, as opposed to Mallory's hypothesis that they arise from the adult hair matrix.

Mummified Epidermal Cysts (So-called "Calcified Epitheliomas"). KING, L. S. (1947). *Amer. J. Path.*, **23**, 29.

A review of the literature relating to "calcified epitheliomata" suggested to the author that several types of these tumours occur. With this in mind he analysed 9 such cases taken from 7,500 consecutive surgical specimens. At the same time 123 epidermal cysts drawn from the same series were also reviewed. The author considers that the term "calcified epithelioma" is misleading and should be abandoned. He suggests that one group of cases be described as "mummified epidermal cysts" and the other group, of which he describes only one, as "basal carcinoma with mummification."

The "Eosinophilic Granulomas" of the Skin. WEIDMAN, F. D. (1947). *Arch. Derm. Syph., Chicago.*, **55**, 155.

The diversity of the disease processes in which cutaneous lesions have the histological features of eosinophilic granuloma is emphasized. The term "eosinophilic granuloma of the skin," not to be confused with eosinophilic granuloma of bone, was used originally for an unclassified cutaneous granuloma in which a dense infiltrate, consisting predominantly of eosinophilic cells, was found; histiocytes, monocytes, plasma cells, and mast cells were also present. It is correct to speak of the condition in the plural, and of a given case as "one of the eosinophilic granulomas of the skin." Five hitherto unpublished American cases are described and most of the published cases are quoted. It is suggested that they fall into two broad categories—idiopathic and symptomatic. All the idiopathic cases are thought to be cutaneous manifestations of one or other of the reticuloses; in all of them some eosinophilia of the blood was present. In the symptomatic cases are included cases of Loeffler's syndrome, erythema elevatum diutinum, orificial tuberculosis, non-specific ulceration, yeast infection, and other unclassified inflammatory conditions. Histologically, a disease known as "syphiloid" of cats can be classified as an eosinophilic granuloma.

Whatever underlying disease may be concerned, it is suggested that eosinophilogenic factors may be responsible for concealing or modifying the typical features of the condition. *G. B. Dowling.*

Eosinophilic Granuloma and its Relation to Xanthomatosis Ossea. (Contribución al estudio del denominado "granuloma eosinofílico" y a sus relaciones con la xantomatosis osea.) SCHAJOWICZ, F., and POLAK, M. (1947). *Rev. Asoc. méd. argent.*, **61**, 218.

The authors describe the relations between three main types of benign reticulo-endothelial hyperplasia of bone: the solitary granuloma, the eosinophil granuloma, and the local or diffuse types of xanthomatosis ossea which may be accompanied by the Schüller-Christian syndrome or which may rarely complicate Letterer-Siwe's disease.

Using classical staining techniques as well as the silver impregnation methods of Rio Hortega, on material obtained from biopsies and excised tumours, the authors studied the relations of the three types of lesions in 8 cases of their own. Radiologically, the lesions were similar in the different cases, there being destruction of bone with at least one focus in the cranial vault. Histologically, the main finding was the hyperplasia of the histiocytic cells, with infiltration by lymphocytes, plasma cells, and polymorphonuclear leucocytes. Eosinophil infiltration was found in lesions from cases differing widely clinically—for example, in a case by simple extirpation, another with the typically chronic lesions of the Schüller-Christian syndrome, and a third with the acute lesions of Letterer-Siwe's disease. In other cases eosinophils were entirely absent from the lesions. In 2 cases of eosinophil granuloma the authors observed follicles of reticulo-endothelial cells, with transitions to multinucleate giant cells, well demonstrated in the lesions by impregnation with ammoniacal silver carbonate. These appearances were reminiscent of those in sarcoidosis and suggested an inflammatory reaction. The intense eosinophil infiltration might represent an allergic type of reaction to a toxin, tuberculous or other. Xanthomatous transformation appeared mostly in the older lesions and was probably not a permanent change, sometimes disappearing again, either spontaneously or after radiotherapy. Because the xanthoma cells were not always found, even in cases with the Schüller-Christian syndrome, the xanthomatous degeneration was thought to be only a stage in the evolution of the histiocytic granuloma, which, possibly with a stage of giant-cell formation, finally goes on to fibrosis. The xanthomatous change would thus be due to a local disturbance of lipid metabolism, secondary to the inflammatory reaction which the authors believe to be the basis of the histiocytic granuloma.

L. P. R. Fourman.

Tumours of Sweat Glands. (Tumeurs sudoripares.) DUPONT, A. (1947). *Arch. belg. Derm. Syph.*, **3**, 275.

The author divides his cases into three categories: (1) sudoriferous tumours whose structure recalls the excretory portion of the sweat apparatus; (2) sudoriferous tumours of the secreting portion; and (3) tumours which, possessing certain characteristics relating them to sweat glands, evolve towards Malpighian epitheliomata.

The histological details of 11 cases are described and illustrated by drawings.

Ewing's Sarcoma of Bone. LICHTENSTEIN, L., and JAFFE, A. L. (1947). *Amer. J. Path.*, **23**, 43.

A study of 17 cases of Ewing's sarcoma of bone is described. Most of the patients were in the second decade of life, 65% being males.

Morphology is described on the basis of four necropsies. Widespread involvement of many bones was often

found in spite of lack of clinical and radiological evidence. Histologically, in all cases crowded cells of uniform size with ill-defined borders were found. They contained little cytoplasm, and their nuclei were large, round, or oval in shape and showed powdery chromatin. Appearances were often variable owing to necrosis and haemorrhage with the subsequent processes of repair.

This description is at variance with Ewing's original description but agrees with that of Oberling.

Metastases were found in the lungs, liver, heart, spleen, kidneys, pancreas, thyroid, and of course in many bones, though it is possible that multifocal origin is the basis of widespread involvement of the skeleton. Lymph nodes were strikingly free from metastases. The breasts, testes, bronchi, gastro-intestinal tract, suprarenals, and sympathetic chain were scrutinized with a view to eliminating the possibility of incorrect diagnosis.

R. B. T. Baldwin.

Endothelial-cell Sarcoma of Liver following Thorotrast Injections. MACMAHON, H. E., MURPHY, A. S., and BATES, M. I. (1947). *Amer. J. Path.*, **23**, 585.

Twelve years after the administration of "thorotrast" for radiographic visualization of the liver a woman aged 71 died suddenly from intra-abdominal haemorrhage from a haemorrhagic lesion of the liver, which the authors regard as sarcomatous. Radioactive deposits of thorotrast were present in the liver, spleen, lymph nodes, bone marrow, adrenals, kidney, and blood-vessel walls. While the diagnosis of sarcoma might be questioned, the case undoubtedly showed widespread damage due to irradiation and haemorrhagic lesions attributable to the thorotrast. *R. A. Willis.*

Multiple Idiopathic Hemorrhagic Sarcoma of Kaposi. Histopathologic Study. SACHS, W., AZULAY, R. D., and CONVIT, J. (1947). *J. invest. Derm.*, **8**, 317.

Haemorrhages, angioblasts ("endothelioid cells" by some authors), and spindle cells are described as constant features of Kaposi's idiopathic haemorrhagic sarcoma. The condition may resemble a simple inflammatory process or an angioma, granuloma, or neoplasm, and the suggestion is made that glomus tumour, pyogenic granuloma, and Kaposi's sarcoma may have a common origin; they may be angioblastomata. Kaposi's sarcoma may be considered as a systemic angiosarcomatosis belonging to this group. *G. A. Hodgson.*

Carcinoma in the Wall of a Lung Cavity. (Das Kavernenkarzinom. Seine Bedeutung für den Arzt und für die Begutachtung). GRÄFF, S. (1947). *Dtsch. med. Wschr.*, **72**, 465.

Six cases of cancer in the wall of a lung cavity in males are reported. These carcinomata are apparently often overlooked even at necropsy. *In vivo* the diagnosis is unlikely to be made. All known cases occurred in tuberculous cavities, originating mostly from the point where the bronchus passes out of the cavity. Microscopically a soft or keratinizing squamous-celled carcinoma was found in all cases. This kind of cancer is seen in the small group of cases in which an external stimulant, here starting from the chronically ulcerated wall of the cavity, is the inciting cause of malignancy.

O. Neubauer.

Bronchogenic Carcinoma: Diagnosis by Microscopic Examination of Sputum and Bronchial Secretions; Preliminary Report. WOOLNER, L. B., and MCDONALD, J. R. (1947). *Proc. Mayo Clin.*, **22**, 369.

The subject of the identification of tumour cells in sputum and bronchial secretions is reviewed, and the writers record their observations in 70 cases in which there were positive findings. They give illustrative examples of these, and conclude that a positive result may be expected in at least 80% of cases of bronchial carcinoma. [In the opinion of the abstracter this is likely to prove an over-optimistic estimate.] The paper points out that a negative result does not exclude the presence of carcinoma, that considerable experience is necessary to distinguish neoplastic cells from others, and that peripheral carcinomata not involving large bronchi usually give negative results. The authors' findings were negative also in cases of bronchial adenoma. *R. A. Willis.*

Invasion of the Internal Mammary Lymph Glands in Carcinoma of the Breast. HANDLEY, R. S., and THACKRAY, A. C. (1947). *Brit. J. Cancer*, **1**, 15.

In 5 unselected cases of carcinoma of the breast a lymph node of the internal mammary chain was removed through the secondary intercostal space at, or shortly after, radical mastectomy. In 4 cases the internal mammary node was histologically proved to contain carcinoma. In only 2 cases were there axillary deposits. The case in which the internal mammary node was free from growth also had no deposit in the axilla. It is suggested that the technique of radical mastectomy might be modified to include removal of the second-space internal mammary lymph node. Microscopical examination of this node might greatly increase the accuracy of prognosis and prove of assistance in post-operative treatment by irradiation. *R. A. Willis.*

Dysgerminoma Ovarii. MAZEL, M. S. (1947). *Amer. J. Obstet. Gynec.*, **53**, 1036.

A review of some of the literature on dysgerminoma ovarii is given, with particular stress on the malignancy of the condition and the need for radical operation even in young women. The author reports a case in a single woman, aged 23.

The Question of Theca-cell Tumours of the Ovary and their Hormonal Function. (Zur Frage der Thekazelltumoren des Ovariums und ihrer hormonalen Funktion.) LIMBURG, H. (1947). *Z. Geburtsh. Gynäk.*, **128**, 186.

Five cases of theca-cell tumours accompanied by post-menopausal hyperplasia of the endometrium are described. In 1 case, 3 years after partial removal of a theca-cell tumour the residue had developed into a simple fibroma. It is probable that all fibromata of the ovary are end-products of theca-cell tumours, analogous to the corpus fibrosum. Oestrogenic ovarian tumours fall into three related groups: (1) pure granulosa-cell tumours, (2) theca-cell tumours, (3) mixed granulosa-cell and theca-cell tumours. Luteinization may take place in any of these. The paper includes an account of a mixed granulosa-cell and theca-cell tumour in a child of 2 years with precocious puberty. *R. Willis.*

Uterine Adenomyosis. Incidence, Symptoms, and Pathology in 1,856 Hysterectomies. HUNTER, W. C., SMITH, L. L., and REINER, W. C. (1947). *Amer. J. Obstet. Gynec.*, **53**, 663.

Adenomyosis was found in 517 cases (27.8%) of 1,856 hysterectomies performed for all causes. The two youngest subjects were each 27 and the oldest (carcinoma also present) 73. While most patients were between 41 and 50, 38 were under 35. Since adenomyosis occurs so often in association with leiomyoma of the uterus it is difficult to evaluate its symptomatology. For this purpose 110 cases of advanced adenomyosis without associated pathology were chosen. Of these, 85 had menorrhagia, 61 dysmenorrhoea, 42 metrorrhagia, 23 pain before periods, 9 dysuria and frequency, 7 pain radiating down legs, 6 bearing-down sensations, and 5 nausea and vomiting. Nearly all had either some menstrual irregularity or dysmenorrhoea. A globoid, slightly enlarged, and tender uterus was usually discovered bimanually. A uterus with leiomyoma and adenomyosis was more tender than one with leiomyoma only.

Granuloma of the Fallopian Tube due to Surgical Glove Talc. Silicious Granuloma. ROBERTS, G. B. S. (1947). *Brit. J. Surg.*, **34**, 417.

An account is given of granulomatous lesions (2 in abdominal scars and 5 in Fallopian tubes) in which the author ascribes the aetiology to talc from surgical gloves. In spite of a latent period of several years (2 to 17) in which the agent appeared to be dormant, the lesion when activated developed within 2 months in 1 case. The silicious nature of the substance deposited, demonstrated in each case with the polarizing microscope, was proved microchemically in only 1 case. Cultures or animal inoculations were not performed, so that tuberculosis may have co-existed. Amongst other clinical features, pelvic pain and infertility are mentioned.

[The author questions a diagnosis of tuberculosis made on histological grounds only. But if these granulomata are due to talc, the danger of the continued use of this form of glove powder must again be considered.]

Histopathological Study of Prostatic Tissue following Endoscopic Prostatic Resection. BARNES, R. W., BERGMAN, R. T., and FARLEY, S. (1947). *J. Urol.*, **57**, 755.

The authors have studied the pieces of tissue removed by transurethral resection of the prostate. They have found that portions removed posteriorly from near the capsule are much more likely to show carcinoma than those from any other site.

Giant Follicular Lymphoblastoma (Brill-Symmers' Disease). PANTRIDGE, J. F. (1947). *Ulster med. J.*, **16**, 46.

This is an account of 7 cases of giant follicular lymphoblastoma. The author considers that the disease is little less than half as common as lymphosarcoma, and that some 27% of lymphosarcomata arise from a preceding follicular lymphoblastoma. It is thought that many cases of lymphoblastoma are unrecognized because early sarcomatous change completely obscures the hyperplastic process.

R. B. Lucas.

The Importance of Malignant Degeneration as a Complication of Chronic Ulcerative Colitis. CATTELL, R. B. and BOEHME, E. J. (1947). *Gastroenterology*, **8**, 695.

Data are presented suggesting that malignant disease may be found to complicate chronic ulcerative colitis in a small but significant percentage of cases if clinical observation is sufficiently prolonged.

Pernicious Anemia and Susceptibility to Gastric Neoplasms. KAPLAN, H. S., and RIGLER, L. G. (1947). *J. Lab. clin. Med.*, **32**, 644.

The authors review the literature on the coexistence of epithelial gastric tumours and pernicious anaemia, and claim that the two diseases develop in the same individuals more often than would be expected on the basis of chance alone.

The precise nature of the relation between pernicious anaemia and gastric carcinoma is not clear. The authors believe that the evidence suggests that the two diseases are probably linked together by some common factor. Possible factors would include hereditary tendencies, achlorhydria, gastritis, and liver therapy.

R. Winston Evans.

Rare Tumours of the Lacrimal Caruncle. (Seltene Geschwulste der Caruncula lacrimalis.) RADNOT, M. (1947). *Ophthalmologica, Basel*, **113**, 270.

Two tumours of the lacrimal caruncle are reported in elderly men. In the first case the tumour was ascribed to an accessory lacrimal gland. In the second the tumour was malignant and appeared to be a sarcoma of a vascular origin.

Pigmented Tumours. REESE, A. B. (1947). *Amer. J. Ophthal.*, **30**, 537.

An exposition and classification of the pigmented tumours of the eye and its adnexa, which may be summarized according to the origin of the melanoblasts as follows:

1. Neurogenic melanoblasts
   (a) Schwann cells } Benign and malignant neuro-
   (b) Naevus cells } genic melanoma.
   (c) Melanoblasts of secondary optic vesicle giving rise to:
      (i) Tumours of pigment epithelium of retina or ciliary body: benign and malignant neurogenic melano-epithelioma.
      (ii) Tumours of muscle of iris: leiomyoma.
      (iii) Tumours of pigment epithelium of iris: dictyoma.
   (d) Melanoblasts of leptomeninges: melanoblastic meningiomata.
2. Ectodermal melanoblasts: pre-cancerous and cancerous melanosis of lids and conjunctiva.
3. Mesodermal melanoblasts of uvea: benign and malignant chromatogenic melanoma.

Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues. MOORE, G. E. (1947). *Science*, **106**, 130.

The author injected 5 ml. of 20% sodium fluorescein intravenously into patients suspected of having malignant neoplasms, and examined the tissue with an ultraviolet lamp emitting rays at about 3,600 degrees. When the interval from injection to examination was between 3 and 8 hours a difference between normal and malignant tissues was observable. In patients subjected to laparo-

tomy for gastric carcinoma, implants of tumour tissue on the peritoneal surface were readily seen, because they fluoresced with a vivid yellow colour. When the tumour tissue was more than a few mm. below the surface no fluorescence was observed, presumably because of the failure of ultraviolet light to penetrate the tissue. Of 46 neoplasms of different organs examined by this technique, 31 exhibited a high degree of fluorescence, 6 only slight fluorescence, and 9 none at all. In each case malignancy was confirmed by histological examination. Carcinomata of the colon, stomach, and breast were less likely to fluoresce, but the best results were obtained with brain tumours. With subcortical lesions the method has been of particular value. Tumour tissue taken from suspected areas by aspiration-needle biopsies was readily recognized by its exaggerated fluorescence in ultra-violet light. A source of confusion in studying fluorescence of abdominal masses is introduced by the fact that areas of oedema and cyst formation may retain the dye for many hours. *R. J. Ludford.*

Meningeal Gliomatosis. A Study of Forty-two Cases. POLMETEER, F. E., and KERNOHAN, J. W. (1947). *Arch. Neurol. Psychiat., Chicago*, **57**, 593.

The authors have collected 42 cases of "meningeal gliomatosis" over a period of 20 years. In 47.6% of cases the primary tumour was a medulloblastoma, in 14.3% glioblastoma multiforme, in 11.9% ependynoma or ependymoblastoma, in 11.9% oligodendroglioma or oligodendroblastoma, in 7.1% astrocytoma, in 4.8% retinoblastoma, and in 2.4% pinealoma. In the 42 cases reviewed there was no instance of metastasis outside the central nervous system. The paper includes a brief review of the subject of glial heteropia, first described by Wolbach in 1907.

Involvement of the Nervous System by Malignant Lymphoma. SPARLING, H. J., ADAMS, R. D., and PARKER, F. (1947). *Medicine, Baltimore*, **26**, 285.

The authors classify malignant lymphomata into 6 types: (1) lymphocytoma, lymphocytic lymphosarcoma, and lymphatic leukaemia; (2) lymphoblastoma, lymphoblastic lymphosarcoma, and lymphatic leukaemia; (3) giant follicle lymphoma; (4) Hodgkin's disease; (5) reticulum-cell sarcoma; (6) plasmocytoma and myeloma. They report 19 cases of involvement of the central nervous system among 118 cases of lymphoma examined at the Mallory Institute of Pathology between 1930 and 1945. In all forms paraplegia due to invasion of the spinal epidural space by growth may occur, and it is the common neurological lesion in giant-celled lymphoma, Hodgkin's disease, reticulo-sarcoma, and plasmocytoma. The authors adduce evidence that the damage to the cord may be due to vascular disturbances. Hodgkin's disease and reticulosarcoma may, however, appear as intracerebral tumours. In leukaemia and lymphosarcoma there may be diffuse infiltration of the meninges and nerve roots, but the most frequent lesion is one or more cerebral haemorrhages. The authors give full clinical and pathological descriptions of the new cases and comment on those previously reported. *J. G. Greenfield.*

On the Origin of Heparin. An Examination of the Heparin Content and the Specific Cytoplasmic Particles of Neoplastic Mast Cell. OLIVER, J., BLOOM, F., and MANGIERI, C. (1947). *J. exp. med.*, **86**, 107.

The authors have estimated the heparin content of two mast-cell tumours in dogs. The first contained 50 times, and the second 1.7 times, as much heparin, weight for weight, as normal dog's liver. The findings support the theory that the mast cells produce heparin.

The Examination of Serous Fluids by the Cell-block Technic. CHAPMAN, C. B., and WHALEN, E. J. (1947). *New Engl. J. Med.*, **237**, 215.

This is an analysis of the results of cytological examination of pleural and peritoneal exudates by the method of sectioning a "false tissue" centrifuge deposit. At the Boston City Hospital during the last 15½ years 833 samples of fluid have been so examined. The material came from 666 patients, in 102 of whom positive results for new growth were obtained. Of 114 cases in which malignant disease was found at necropsy or biopsy, cell-block preparations were positive in 47. The diagnostic criteria were rather more stringent than those often employed elsewhere. For a positive diagnosis the authors require that fully or partly formed acini or sheets composed of cells showing definite evidence of anaplasia should be present. *D. H. Collins.*

Significance of the Cell Types of the Human Islets of Langerhans in Islet Function and Diabetes Mellitus. (Die Bedeutung der Zelltypen des menschlichen Inselapparats für Inselfunktion und Diabetes mellitus.) TERBRUGGEN, A. (1947). *Klin. Wschr.*, **24/25**, 434.

Employing a technique similar to that used in animals by Lane and Bensley, the author has examined the number of α- and β-cells in the pancreas of normal and diabetic men. There is in diabetic subjects both a relative and an absolute reduction in the number of β-cells in the islets of Langerhans and this reduction seems to be responsible for the disease. The ratio of α-cells to β-cells in normal adults has been found to be from 1 : 3 to 1 : 5. In juvenile diabetic patients (those who died before the age of 40; 8 cases) the ratio was mostly from 1 : 1 to 1 : 2 and was in no case higher than 1 : 3. Results in diabetic subjects over 40 (18 cases) were not so clear. Here the results are complicated by other changes in the islets, especially hyaline degeneration and sclerosis, but in islets not hyalinized and not sclerosed the same diminution of β-cells has been observed. In hyperinsulinism a relative diminution of the β-cells has also been observed; this may be explained as an adaptation. In adenomata of the islands very few α-cells are seen. The β-cells obviously do not originate from the α-cells; the latter may represent a stage of rest or be regarded as a special kind of cell. *O. Neubauer.*

The Histopathology of Acute Mumps Orchitis. GALL, E. A. (1947). *Amer. J. Path.*, **23**, 637.

During an epidemic of mumps among adult males, orchidotomy was practised for acute mumps orchitis and biopsies of testis from 75 such cases were taken, the majority within 48 hours of onset and none later than the fifth day. Necropsy material was obtained from 1 patient who died of pulmonary embolism 11 days after the onset of orchitis. Stages of degeneration and inflammation are described. Complete atrophy is unusual, although permanent focal damage may result. Owing to the interval of 11 days between onset of orchitis and death, the lesions were more intense and the processes of repair became more apparent; fibrosis and hyaliniza-

tion of the lamina propria of the affected tubules were beginning, macrophages were more numerous, and polymorphs were scarce. Several groups of tubules showed actively proliferating normal epithelium.

Specimens of epididymis revealed interstitial changes similar to those in the testis. The epithelium seemed normal, but the lumina were filled with plugs of debris coming from the damaged tubules.

Dysontogenetic Pituitary Cysts. (Pituitary Cachexia in Childhood.) BARR, H. S. (1947). *Arch. Dis. Childh.*, **22**, 118.

Two cases of pituitary cachexia in childhood were due to large colloid cysts of Rathke's cleft with pressure atrophy of the anterior lobes. The designation "dysontogenetic pituitary cysts" for this type is proposed. The reasons are pointed out why the designation "tumours of Rathke's pouch" for carniopharyngiomata is considered to be a misnomer. The pituitary cachexia was associated with syringomyelia in one case and with fibrocystic dystrophy of the pancreas in the other. The possibility of a causal link between the latter and the pituitary disease is discussed.—[From the author's summary.]

Hypercalcemia and Idiopathic Hyperplasia of the Parathyroid Glands in an Infant. PRATT, E. L., GEREN, B. B., and NEUHAUSER, E. B. D. (1947). *J. Pediat.*, **30**, 388.

An infant was studied from the age of 15 weeks until its death at 10 months. The leading symptoms were weakness, hypotonia, lethargy, anorexia, and malnutrition. Investigations revealed a constantly high blood calcium with low urinary calcium excretion and deficient renal function. Radiographs of bones showed some hypocalcification, and biopsy revealed fibrosis of the marrow spaces.

At necropsy there was very slight enlargement of the parathyroids, which microscopically showed lack of stroma, great cellularity, and increase in chief cells and transitional water-clear cells. Some nephrocalcinosis and a calcium plaque in the aorta were seen, but no other metastatic calcification was observed. The bones showed osteitis fibrosa. No satisfactory explanation is offered for the apparently unique association of hypercalcaemia with chief-cell hyperplasia.

N. M. Jacoby.

Myelolipoma of the Adrenals. Report of Seven Cases. GIFFEN, H. K. (1947). *Amer. J. Path.*, **23**, 613.

Seven examples of heterotopic development of bone marrow in the adrenal glands are described, 4 with yellow and 3 with red marrow; all were in middle-aged or elderly people, none of whom showed significant anaemia. The cause and histogenesis of the change are obscure.

R. A. Willis.

Polycystic Disease of the Kidney. A Review. LAMBERT, P. P. (1947). *Arch. Path.*, **44**, 34.

It has been proved by serial sections that the cysts in neonatal cases do not communicate with the pelvis; these patients survive for only a short time. Although the adult type is clinically so different (in that the patient often lives to middle age), it has generally been assumed that the cysts were also closed in this type. Cysts in adult cases had not been studied in serial sections because of their size, but this has now been done in 5 adult cases with unexpected results.

Cysts are classified as: (1) Glomerular cysts: these have a normal glomerular tuft and granular content. They never communicate with the pelvis in either adult or neonatal cases. (2) Tubular cysts: these have a colloid content. They communicate with the convoluted tubules or loops of Henle, and in adult cases the nephrons thus affected generally communicate with the pelvis. (3) Excretory cysts, connected with the collecting tubules. All the above may be simple or complex. (4) Cysts of the calices.

The author produces evidence that the nephrons connected with tubular and excretory cysts function normally, thus accounting for survival of these cases into middle age. The author discredits previous theories of the genesis of the disease, and it is clear that non-union of ureteric tubules and nephrons will not account for the formation of cysts which communicate with open tubules.

D. M. Pryce.

Acute Liver Necrosis in Fulminating Infectious Hepatitis. TAYLOR, H. E. (1947). *Amer. J. clin. Path.*, **17**, 314.

Four fatal cases of fulminating liver necrosis in infectious hepatitis, homologous serum jaundice, and arsenotherapy jaundice have been presented. The length of illness was 4 or 5 days. The essential lesion was an extremely rapid diffuse necrosis and disintegration of the parenchymal liver cells. No normal cells were found in the many blocks examined. There was an associated meningo-encephalitis in 2 cases.—[From the author's summary.]

The Pathology of Epidemic Hepatitis. MALLORY, T. B. (1947). *J. Amer. med. Ass.*, **134**, 655.

Biopsies of liver from 160 patients in various stages of the disease, and necropsy material from 296 cases have been studied. Histological stages of the disease are described.

Studies of Gaucher Cells. (Etudes sur la "cellule de Gaucher.") FRANCO, S., and WOLMAN, M. (1947). *Schweiz. Z. Path. Bakt.*, **10**, 621.

A staining method is described for the demonstration of keratin in the spleens of patients who died from Gaucher's disease. Frozen sections of formalin fixed tissue are treated with ether and acetone to remove the fat; after the sections have been exposed for 30 seconds to boiling water at *p*H 4 they are stained with Sudan III. The method is specific for cerebrosides, and in the cases described for keratin.—[From the author's summary.]

The Cutaneous Lesions in Acute Meningococcemia. A Clinical and Pathologic Study. HILL, W. R., and KINNEY, T. D. (1947). *J. Amer. med. Ass.*, **134**, 513.

This paper describes the pathogenesis of the cutaneous lesions in acute meningococcaemia, through the stages of endothelial damage, inflammatory necrosis, and thrombosis. The first is responsible for the purpuric rash sometimes proceeding to local gangrene. In the early stages histological examination may show desquamation of vascular endothelial cells, which elsewhere may exhibit phagocytosis of cocci. Invasion by polymorphonuclear leucocytes follows, many cells displaying active phagocytosis. Platelet thrombi, becoming organized in the later stages, were most commonly found in deeper vessels of the cutis, though the superficial

capillaries were also involved. The stained sweat glands were abnormally pale, their cytoplasm appearing swollen or vacuolated, the result of interference with the blood supply of the gland. The hair, muscles, and nerves showed similar degenerative changes and focal necroses were seen in subcutaneous fat. Similar vascular lesions were found in many other organs and are by no means limited to the skin. The authors suggest that the formation of thrombi may cause thrombocytopenia and thus lead to purpura. They say that in 2 cases without purpura the platelet count was normal. Other workers, however, have found normal platelet counts in patients showing a purpuric rash. *R. H. D. Short.*

Transitory Diabetic Syndrome associated with Meningococcic Meningitis. FOX, M. J., KUZMA, J. F., and WASHAM, W. T. (1947). *Arch. int. Med.*, **79**, 614.

In 233 cases of meningococcal meningitis, 1 in 4 showed transient diabetes mellitus. Fortunately, if the septicaemia is well and promptly treated the diabetic syndrome subsides. In 5 cases a wrong diagnosis was made, and the patient, who was in fact suffering from meningococcal septicaemia, was deemed to be in a state of diabetic coma and treated accordingly. During such a mistaken course of treatment irreparable damage may be done to the tissues and viscera. Seventeen cases were examined at necropsy, but the findings do not sustain the contention that glycosuria in meningitis may be due to damage to the pituitary as all the viscera, especially the liver, thyroid, spleen, pancreas, and adrenals, showed damage. *G. F. Walker.*

Histopathologic Changes Associated with Human Poliomyelitis. SCHEINKER, M. (1947). *Arch. Neurol. Psychiat., Chicago*, **57**, 565.

The results of studying 6 cases of acute anterior poliomyelitis support the now generally held view that this disease of the central nervous sytem is not as focal as was formerly thought but can be widespread in its incidence. The author finds no direct parallelism between the neuronal destruction and the inflammatory reactions in the mesoderm. Cellular infiltration, for instance, is most intense in the medulla and midbrain, especially in the vicinity of the nucleus ambiguus, substantia nigra, and aqueduct, in all of which places neuronal destruction is slight compared with that in the anterior horns of the spinal cord. These cases, in that they exhibited massive inflammatory changes in the region of the vagal nuclei, tend to support the view that the virus may spread from the upper part of the alimentary tract to the brain through the fifth, seventh, ninth, and tenth cranial nerves. *W. H. McMenemy.*

A Clinico-Pathological Report of Unusual Cases of Chronic Encephalitis. ROSANOFF, W. R. (1947). *J. Neurol. Neurosurg. Psychiat.*, **10**, 65.

Three cases of encephalitis are described. All were insidious in onset and progressive over periods varying from 4 months to 5 years. They were characterized by intense inflammatory changes widely distributed throughout the brain. Case 1 was distinguished by a particularly severe involvement of the cerebral white matter which closely resembles the "leuco-encephalite sclerosante subaigue" of van Bogaert. The second had a positive iron reaction. In the third there was the combination of diffuse and focal cystic lesions characteristic of some of the extra-European types of epidemic encephalitis.—[From the author's summary.]

Deposition of Iron in Paraventricular Areas of the Human Brain in Hemochromatosis. CAMMERMEYER, J. (1947). *J. Neuropath. exp. Neurol.*, **6**, 111.

The author studied the depcsition of iron pigment in the brain in a long-standing case of haemochromatosis and compared his findings with the results of injections of vital dyes by Wislocki, King, and others. He concludes that the deposition of iron in the paraventricular areas of the brain is related to structural and functional peculiarities of the blood vessels in these areas.

J. G. Greenfield.

Studies on Typhoid Pneumonia. Communication I. Pneumonia Types in Typhoid Fever (in Russian). LAZOVSKY, Y. M., KUDRAVITSEVA, E. N., and MELNIK, E. G. (1947). *Klin. Med., Mosk.*, **25**, 35.

The authors examined 35 cases of pneumonia in typhoid fever and concluded that although 15 were of the hypostatic type, 20 showed changes which they considered specific for typhoid pneumonia and which resembled the lesions found in the alimentary canal. Whole lobes were often involved, and pneumonia was bilateral in 13 cases.

Pulmonary Vascular Lesions in Silicosis and Related Pathologic Changes. GEEVER, E. F. (1947). *Amer. J. med. Sci.*, **214**, 292.

An analysis is made of the vascular lesions in 43 unselected and consecutive cases of pulmonary silicosis. In 26 there was hypertrophy of the right ventricle attributable to the silicosis alone. Changes in the lung parenchyma are also described.

Lymphogranuloma Venereum. A Histologic Study of the Primary Lesion, Bubonulus, and Lymph Nodes in Cases Proved by Isolation of the Virus. SHELDON, W. H., and HEYMAN, A. (1947). *Amer. J. Path.*, **23**, 653.

The authors suggest that the histological appearances of biopsy specimens are sufficiently typical to enable diagnosis to be made with reasonable certainty. Their observations are based on examination of material obtained from 12 cases of the disease in which the diagnosis had been confirmed by large numbers of tests, both clinical and laboratory. In 8 of these cases absolute proof was obtained by isolation and identification of the virus from material derived from the lesions.

The earliest lesion is the formation of foci of large mononuclear cells in the adventitia of the small blood vessels or, in the case of the lymph nodes, in the cortex just beneath the marginal sinus. The proliferation proceeds to involve all the coats of the blood vessels and eventually obliterates their lamina by compression, and *without* the vascular endothelial proliferation or thrombosis usually stated to take place. Similar changes affect the sinuses and small capillaries in the lymph nodes, so that small granulomata are produced. Their centres undergo ischaemic necrosis and numerous polymorphonuclear leucocytes appear. Thus small abscesses are formed which by fusion form larger ones and, if the lesion be near the skin, as in the case of the primary lesion particularly, the epidermis breaks down and an ulcer is formed. Peripherally the abscess is surrounded by a ring of mononuclear cells, outside which a few giant cells may be found together with plasma cells and

lymphocytes in small numbers and a very occasional eosinophil leucocyte. Fibrosis was scarcely noticeable in the acute stages, and was present only to a small degree in the node removed from the patient in whom the disease had been cured. The authors found the eosinophilic intracytoplasmic inclusions, or "gamma bodies," only after necrosis had taken place, and believe that they are merely debris phagocytosed by the mononuclear cells and bear no relation to the virus of lymphogranuloma. No elementary bodies were found.

Temporal or Giant-Cell Arteritis. ROBERTSON, K. (1947). *Brit. med. J.*, **2**, 168.

Details are given of 4 cases of temporal arteritis met with in a period of a little more than a year, 2 of which were proven by biopsy. The patients were aged 65 (2), 73, and 65. In 3 cases the erythrocyte sedimentation rate was high for a considerable time. The blood urea is normal. Progressive secondary anaemia and mild leucocytosis were present. The disease is self-limited and rarely fatal, and treatment is entirely symptomatic. It differs from periarteritis nodosa in that the latter attacks younger people; the visceral vessels in periarteritis nodosa suffer severely, the blood urea is almost always raised, and the mortality from the disease is high.

S. Oram

Ostropetrosis : Albers-Schonberg Disease (Marble Bones). Report of a Case and Morphologic Study. PINES, B., and LEDERER, M. (1947). *Amer. J. Path.*, **23**, 755.

The authors review the literature and present details of the necropsy and histology of the bones in an infant. The original paper should be consulted.

Nodular Inflammatory and Degenerative Lesions of Muscles from Four Hundred and Fifty Autopsies. CLAWSON, B. J., NOBLE, J. F., and LUFKIN, N. H. (1947). *Arch. Path.*, **43**, 579.

In small biopsies of skeletal muscle in rheumatoid arthritis Steiner *et al.* found chronic inflammatory foci composed mainly of lymphocytes in each of 9 cases. They also noted various degenerative lesions in the muscle fibres. They found no similar lesions in a control series of 196 necropsy specimens, and considered them specific and indicative of an infective aetiology. The present authors found lymphocytic foci in 39% and degenerative lesions in 25% of 44 clinical cases of rheumatoid arthritis. They noted lymphocytic foci in 26% and degenerative lesions in 42% of 450 controls. The incidence increased with age. The muscles most commonly affected were the diaphragm and sacro-spinalis. Although more common in association with rheumatoid arthritis, the lymphocytic infiltrations are not specific.

D. M. Pryce.

Deaths Following Use of Abortifacient Paste. Report of Two Cases. KULKA, W. (1947). *Amer. J. clin. Path.*, **17**, 723.

The necropsy findings are described in 2 patients in whom death followed the injection of an abortifacient paste. In 1, death occurred 2½ months after the attempted abortion. Necrosis of the uterine wall, parametrial abscesses, and generalized peritonitis were found. The second patient died suddenly of pulmonary embolism within a few hours after an attempt to cause abortion by intrauterine injection of the paste. The emboli were composed of fatty material and particles from the damaged chorionic villi. There was necrosis of some of the villi and vessels of the uterine wall but no apparent changes in the embryo or the embryonic sac. The presence of a pasty material was demonstrated by the technique used as a routine for the demonstration of fat but the staining reaction was different from that of fat.—[Author's summary.]

Sudden Deaths of Infants Allegedly Due to Mechanical Suffocation. WERNE, J., and GARROW, I. (1947). *Amer. J. publ. Hlth*, **37**, 675.

During the past 15 years 167 consecutive cases of infants ordinarily certified under the heading of "accidental mechanical suffocation" have been investigated by the authors. In no case did they find that an infant had been suffocated. In 43 cases necropsy alone was sufficient to determine that death was due to a natural cause, mainly upper respiratory tract infection. In the remaining 124 cases histological study showed that fulminating respiratory disease was the most likely explanation for death in all except 15, in 6 of which no tissues were available for microscopical examination. An examination was made of 67 other infants who had died suddenly in circumstances in which there could be no possible allegation of smothering. In these, acute respiratory disease was the usual cause of death.

OBITUARY

EDWARD FFOLLIOTT CREED

It is with sorrow that we have to record the sudden leath of Edward ffolliott Creed at the age of 54 luring his convalescence from a severe illness. He vas one of the original members of the Association of Clinical Pathologists. He had succeeded W. D'Este Emery as director to the Pathological Department at King's College Hospital in 1921. There had been several months' delay in filling the post, perhaps because of doubts in the minds of his colleagues due to his relative youth and inexperience. These doubts were soon set at rest and the Department flourished under his care.

Creed was largely responsible for what was, in the early nineteen-twenties, an innovation in teaching hospitals—namely, the creation of a post of resident assistant clinical pathologist. His intention was probably twofold: first, to introduce the budding physician or surgeon to a working knowledge of laboratory methods in clinical medicine, and help him not only to understand clinical medicine the better but also, and perhaps more important, to appreciate the value and limitations of laboratory methods; secondly, to provide the early training ground for the clinical pathologist of the future. This was a very necessary provision, because up till then almost the only way for one who wished to become a clinical pathologist was to enter a university pathological laboratory as a junior demonstrator and go through a training which, though of great value educationally and scientifically, often failed to provide all that was needed for one who would be in charge of a clinical laboratory. The resident pathologist also learnt to bridge the gap between bedside and laboratory, on the one hand helping his colleagues the house physician and house surgeon to use the laboratory more efficiently and less wastefully; and on the other, keeping the laboratory closely in touch with what was going on in the wards. Creed felt strongly the constant need for this close association, and resisted all measures which threatened it, such as the siting of laboratories at distances from the wards. *Pari passu* he was not attracted by postal pathology,

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and he tolerated it only because of the exigencies of the time.

H. A. Osborn was the first, Terence East the second holder of the new post. I was the third, and there have since been a succession of trainees who are now scattered over the country, many of them running laboratories; some, practising in other fields, now

know that their spell in clinical pathology under his guidance was time well spent.

To one closely in touch with him over the last quarter of a century, an outstanding feature in his character was his humility and tolerance, shown even to the callow and the ignorant. He remained free from the accretions of dogma and self-assurance which are apt to clog the minds of teachers in their fifth or sixth decades. His humility was genuine, and prevented him from publishing work which ought to have been published long since—for example, his careful and critical elaboration and assessment of the technique of blood fragility estimation, which he did not publish for some fifteen years. His humility was not due to any lack of critical faculty; he was quick in finding weak spots in an argument. Some of us can recall the quiet way in which after a meeting he could on occasions tear to pieces an elaborate and convincing thesis.

He was a pupil of Georges Dreyer, of Oxford, and was essentially a clinical pathologist. His interest always lay in the practical application of pathology to medicine. He was too occupied in perfecting techniques for routine work to tackle more basic problems. He upheld the tradition that the pathologist should be constantly at the bedside, and as his interest lay in clinical problems his heart was in his work.

A careful and patient teacher to the many, to the few who knew him well his loss will be keener and more personal. They will miss a delightful and sympathetic companion, perhaps a trifle reserved, but one whose conversation, full of wisdom, was lit by frequent shafts of whimsical humour.

C. H. WHITTLE.

We regret to record the death of Professor James McIntosh, Director of the Bland-Sutton Institute of Pathology, Middlesex Hospital, and Professor of Pathology in the University of London.